Acta OTO-LARYNGOLOGICA

VOL 82 · JULY–DECEMBER 1976 · No 1–6

ACTA OTO-LARYNGOLOGICA 82 1–6 1976 GREV MAGNIGATAN 18 11455 STOCKHOLM

Almqvist & Wiksell, Uppsala 1976

AUTHOR INDEX

SUBJECT INDEX

Acta Otolaryngol 82 1-10, 1976

ON TUBAL FUNCTION IN PRESENCE OF CENTRAL PERFORATION OF DRUM IN CHRONIC OTITIS MEDIA

Clinical Methods and Preoperative Analyses – 132 Cases

L Andreasson and A Ivarsson

From the Department of Otolaryngology Malmo General Hospital Malmo Sweden

(Received August 4 1975)

stract Opinions differ on the value of preoperative ting of tubal function The results vary widely but so r no comparative investigation between different tubal nction tests seems to have been published On 132 ears ith central tympanic perforation 9 of which were aumatic and the remaining following chronic otitis edia tubal function was studied with a closed man metric method and open flow volume method which ieasures the tubal volume passage The results of the losed and open techniques agreed in 96% The open iethod is more sensitive but the closed method is easier 0% of the total material had aspiration capacity i e ould neutralise middle ear pressures which is better than arlier results Toynbee s test is a good screening test and when positive it means good tubal function A close cor elation was found between tubal function and the volume of the air cell system as an effect of the systemic mucosal damage

Gas in the middle ear is continuously being absorbed with a consequent reduction of the pressure The intratympanic and atmospheric pressures are equalized by opening of the Eustachian tube in association with the act of swallowing Investigations of gas absorption during swallowing on intact ears have been published by Ingelstedt & Jonson (1967), Riu et al (1966), Elner et al (1971*a*, *b*), and others

Advances made in reconstructive surgery of the middle ear since the early 1950s have aroused increased interest in the function of the Eustachian tube in chronic otitis media Postoperative impairment of the function of the Eustachian tube can spoil the effect of an otherwise successful operation Preoperative estimation of the function of the Eustachian tube therefore soon became a routine measure for judging the prognosis in a given case In the beginning well known simple tests were used such as Politzer's test and Valsalva's manoeuvre, which give information about the patency of the tube The aspiration-deflation method described by Flisberg et al (1963) is a more physiological test because in this test negative pressures are applied to the middle ear, and it now seems to be the method most widely used (Miller 1965, Flisberg, 1966, Holmquist, 1968, Siedentop, 1968, Ekvall, 1970, Palva & Kärjä, 1969, Sharp, 1970, Niwa, 1972, and others)

However, the investigations on record differ from one another in composition of the material, test pressure applied, and the classification of tubal function, differences which make direct comparison difficult Moreover, some workers question the value of preoperative testing of tubal function Some investigators (Miller & Bilodeau, 1967, Holmquist, 1968, Siedentop, 1968, MacKinnon, 1970, Niwa, 1972) have found the results of operation to be better in the absence, than in the

This study was supported by the Swedish Medical Research Council (Nos B72 17X 99-08 B73 17X 99-09) and the Delegation for Applied Medical Defence Research (Nos U87/1972 U95/1973)

preoperative impairment of tubal function, while Sharp (1970) is of the opinion that preoperative testing gives no valuable information, and Smyth et al (1971) claim that it "can safely be excluded"

The aim of this investigation was twofold, viz to compare different methods of testing tubal function in a well defined material with central drum perforation in order to find out the most reliable and the simplest test for clinical purposes and, second, to compare the findings in the present material with those in an investigation of healthy ears under the same testing conditions (Elner et al, 1971*b*) in an endeavour to assess to what extent they differ *The investigation was included as a routine* procedure in part of preoperative studies of patients with chronic otitis media on the assumption that the mucosal damage is reflected both in volume reduction of the air filled spaces and in impairment of tubal function (Andreasson, 1973, 1975)

METHODS

Different methods were used for estimating the ability of the Eustachian tube to equalize the intratympanic pressure and the atmospheric pressure The methods are based on recording of the equalization of pressure (closed method), on one hand, and on recording of the flow-volume of air passing through the open tube (open method) during swallowing, on the other hand

Closed method (pressure recording)

This method was first described by Flisberg et al (1963) A polyethylene catheter with a cuff connects the middle ear hermetically with a transducer *EMT33 (Siemens Elema AB)* through the perforation in the drum (Fig 1) With the 3 way stopcock in position *b* a desired pressure is applied from the pressure maintaining system (Fig 3) In cock position *c* the middle ear pressure is affected only when the Eustachian tube is opened

The volume of the equipment in this position *c* 1 ml Transducer data pressure ran[ge] ± 40 cm H_2O, *max linearity error* <[…] within the pressure range, pressure memb[rane] deviation 0 05 μl/cm H_2O Pressure is [re]corded on an ink jet recorder M 800 (Sie[mens] Elema AB) Before and after every test [the] equipment was calibrated with the test p[res]sure ± 5 and ± 10 cm H_2O and controlled [with] *a horizontal alcohol manometer Reading* [ac]curacy of the alcohol manometer ± 0 05 [cm] H_2O

Open method (flow volume recording)

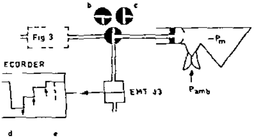
The principle of the method is based upo[n an] open connection between the ear canal [and] the external pressure, P_{amb}, through a fl[ow-]volume meter (Fig 2), and a pressure m[ain]taining system (Fig 3) The flow-volume [me]ter records the air flow through the Eustac[hian] tube (V_t) as well as that (V_p) caused by c[om]pression/decompression of the air in the m[…]

system (P_m) The air flow signals from the [dif]ferential pressure transducer EMT 32 ([Sie]mens Elema AB) are *integrated electronic*[ally] to obtain a signal corresponding to the vol[ume] passing through the tube during swallow[ing] $\Delta V_t = \int V_t \times dt$ The intratympanic pressure, [P,] and the volume, ΔV_t, are recorded on an [ink] jet recorder M 800 (Siemens Elema AB)

The equipment was calibrated before [and] *after use with an airtight syringe giving* [the] test volumes of 10 and 20 μl Reading [ac]curacy of the syringe ± 0 1 μl For data [see] Rundcrantz 1969

Pressure maintaining system

The intratympanic pressure was raised a[nd] *lowered with the equipment illustrated in F*[ig] 3 It consists of a fan and a flow regula[tor] (FR) described by Jonson (1969), which [in] spite of the variation in r p m creates a co[n]stant air flow of 0 5 l/sec By series connecti[on] of one of the capillaries exposed to the atmo[s]pheric pressure, constant over- or underpre[s]sures of 2 5 or 10 cm H_2O can be produce[d]



Equipment for pressure recording

desired pressure is applied to the middle
it *a* The range of variation of an adjusted
sure is ±2%

ssure generator and measuring device
hinopharynx

order to apply constant pressures to the
nopharynx an equipment earlier described
Ingelstedt et al (1963) was used The rhino
arynx pressure, P_{rh}, is recorded in this test
well as in Valsalva's manoeuvre and Toyn
's test by a pressure transducer EMT 35
emens Elema AB)

Transducer data Pressure range ±400 cm
O Max linearity error <2% within the
essure range

ie cuff system

he middle ear is connected with one of the
o equipments by a catheter 54 cm long and
2 mm in inner diameter It is fitted with a
am rubber cuff with a thickness of 3 mm and
ith an individually selected diameter (5–10
m) The cuff is placed into the bony part of
e ear canal where it is fixed airtight with
opcock grease

Performance of Test

The catheter with an appropriate cuff was ap-
plied in the ear canal The cuff was examined
nanometrically for leakage as well as for false
esponses during movements of the jaw The
test required about 1 5 hours and the different
tests were always done with the patient sitting
and in the following order

Open method (flow-volume recording)

(1) Politzer's test to confirm any organic obstruction of the Eustachian tube and to expel mucus, if any

(2) Toynbee's test The pressure in the rhinopharynx was recorded through a nasal olive placed in one of the nostrils When the tube was open, the volume passing through the Eustachian tube was recorded The test was done three times

(3) Aspiration-deflation test Test pressures of ±2, ±5 and ±10 cm H_2O were intermittently applied to the test ear for a few seconds, during which the patient was instructed to swallow At each pressure gradient the volume that passed through the tube was recorded during 10 swallowings within a 3 min period The patients could swallow dry or with a small sip of water, if desired

(4) Graded constant pressures in the rhinopharynx A nose mask connected to the pressure producer was pressed airtight over the nose During swallowing a magnetic valve connected with the pressure producer was opened thereby building up a graded constant over- or underpressure in the rhinopharynx The test was done at ±10 and ±25 cm H_2O with 10 endeavours to equilibrate at each pressure gradient

(5) Valsalva's manoeuvre This was done after the patient had received careful instructions The pressure in the rhinopharynx and the passage, if any, of air were recorded simultaneously and the pressure, if the tube was patent, was noted (forcing pressure)

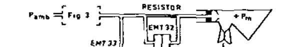


Fig 2 Equipment for flow volume recording

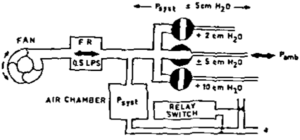


Fig 3 Pressure maintaining system

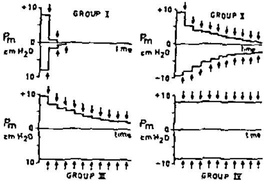


Fig 4 Classification of tubal function according to to equalize an intratympanic pressure of ±10 cm H the extratympanic pressure Arrows denote acts of lowing

Closed method (pressure recording)

(6) Aspiration deflation test This was done at a test pressure of ±10 cm H_2O The patient swallowed until the pressure gradient was equilibrated or 10 times during 3 min at most, after which any residual pressure showed the functional reduction The test was done at least three times

MATERIAL

The clinical material consisted of 126 patients (64 women and 62 men) with central perfora tion of the drum in 132 ears The age distribution and classification can be seen in Table I

In nine ears the perforation was recent and ue to trauma The remaining 123 ears were due to chronic otitis media, which the patients had had for 1–55 years Only patients with ears that had been dry for at least two months and who showed no sign of upper respiratory tract infection were accepted All ears were in spected under an operating microscope and only ears with a clear central perforation without signs of cholesteatoma were included in the material All ears with impaired tubal function (39) as well as some with good function (16) were re tested after an interval of at least 2 weeks 5 cases were intentionally tested during upper respiratory tract infection and after wards in a non infectious interval

RESULTS

Closed method (pressure recording)

Aspiration-deflation As the same test proce dures and numbers of swallowings were used in this investigation and that of Elner e (1971*b*) of healthy ears with an intact d the same classification of tubal function used (Fig 4)

Group I
equilibrates intratympanic pressure atmospheric pressure completely,

Group II
equilibrates but with residual pressure in middle ear,

Group III
equilibrates relative overpressure but not derpressure,

Group IV
no equilibrating capacity at all

Table II shows the distribution of patie among the tubal function groups No stat

Table I *Distribution of the material accor to age and etiology*

Age	Chronic otitis media	Traumatic perforation
8–10	5	
11–20	18	1
21–30	33	2
31–40	19	3
41–50	22	1
51–60	21	2
61–70	5	
Total	123	9

II *Classification of tubal function ac-
ng to Elner et al (1971 b)*

on	Chronic otitis media		Traumatic perforation	
	No of ears	%	No of ears	%
	69	56 1	6	66 7
	17	13 8	1	11 1
	12	9 8	2	22 2
	25	20 3	–	–

significant difference (χ^2 analysis) was
d between cases of chronic otitis media
cases with a traumatic perforation This
of difference might have been due to the
ll number of ears with traumatic perfora
s
ouble investigations at an interval of at
t two weeks showed good agreement in
% (48 out of 55 ears tested) but noteworthy
erences in the remaining 13% (5 cases of
up IV and 2 cases of group III changed to
up II) The 5 cases examined during upper
piratory tract infection belonged to group II
group III (3) and group IV (1) When not
ected all the ears showed good tubal func
n i e group I

pen method (flow volume recording)

able III gives the number of ears with at least
ne passage at different test pressures Pa
ncy was more common when the pressure
adient was rising Moreover patency was
ore common when the intratympanic pres
ure was increased than when it was de
reased even though the pressures were ap
lied intermittently

Table III *The percentual number of cases where the Eustachian tube was patent on at least one occasion at different pressure gradients*

Middle ear pressure cm H_2O	+2	+5	+10	2	5	10
Ears with tubal passage in % of total material	59	75	84	56	69	74

With the open method the tubal passage capacity can be graded as the number of suc cessful passages as well as the order of the volume Table IV compares open and closed methods as the number of tubal passages dur ing 10 swallowings at intratympanic ear pres sures of ± 10 cm H_2O correlated to tubal func tion groups With but few exceptions the re sults obtained with the closed and open methods show good agreement Thus the tube was patent as tested with the open method in group IV at +10 cm H_2O in 5 cases and in group III at −10 cm H_2O in 3 cases where the closed method showed no equilibration Only one case (group III at +10 cm H_2O) showed the opposite result, i e equilibration with the closed but not with the open method

The volume of the air passage varied widely within and between tubal function groups Fig 5 shows the mean volume of the air passage (ΔV_{tmean}) tested at ± 10 cm H_2O in group I The range of variation was wide 12–500 μl at posi tive and 16–450 μl at negative intratympanic pressures Most of the dots in Fig 5 are to the right of the line indicating that the air volumes were larger when the intratympanic pressure was positive than when it was negative Groups II–IV showed successively reduced air passages

Politzer s test This was invariably positive i e no tube was occluded

Toynbee s test In 60% of the whole material the Eustachian tube was patent (Table V) The

Table IV *Direct comparison between open and closed method*

Tubal function groups closed method (no of ears)		Tubal patency during 10 swallowings (open method) No of ears +10 cm H_2O	No of ears 10 cm H_2O
I	75	75	75
II	18	18	18
III	14	13	3
IV	25	5	2

Table VII *Comparison between different investigations concerning aspiration capacity number of swallows and test pressures (a) ears with normal mucosa out of a larger materi (b) ears with central perforation and intact ossicles out of a larger material*

Invest	No	Pos asp (%)	No of swallows	Test pressure (cm H_2O)
Miller (1965)[a]	54	59	1–36	25
Flisberg (1966)	96	42	Not given	2–40
Miller Bilodeau (1967)	91	63	Not given	2 5–25
Siedentop et al (1969)[a]	*144*	*44*	*1–>6*	*25*
Holmquist (1968)	*217*	*48*	*Not given*	*20*
Ekvall (1970)[b]	21	38	≥10	2–50
Sharp (1970)	40	7 5	1–30	10–30
Andreasson et al 1975	123	70	1–10	10

chronic otitis media (Flisberg, 1966) The differences are thus much larger in the presence *than in the absence of disease Zollner and* Elner et al were of the opinion that positive Toynbee s test means normal tubal function which was confirmed in this study also for chronic otitis media

When testing with graded inflation of the rhinopharynx Elner et al found no difference in the flow at positive or negative rhinopharyngeal pressures The flow also showed good correlation with the tubal function grouping in their material There was no such good correlation in this study, especially not for group III (Table V) The method has no advantages in practical clinical work

Positive Valsalva s manoeuvre showed a poor correlation with the tubal function groups as for healthy ears in the study of Elner et al The Valsalva manoeuvre only shows the forcing capacity of the Eustachian tube and is in no *sense an active function test In the materials* of the above authors Valsalva's manoeuvre was positive in all the patients with negative aspiration capacity 7%, groups III and IV *When these persons were studied with the use* of the spontaneous gas diffusion for producing negative middle ear pressure, equilibration was achieved *by spontaneous blowing of their* nose and not by swallowing The Valsalva manoeuvre can thus predict whether and how the patient might equilibrate the pressure in the middle ear in the postoperative period

Comparison with earlier investigations of chronic otitis media

As mentioned earlier, comparisons betwee earlier series of chronic otitis media and th present material are difficult because of differ ences in the composition of the materials dif ferences in initial pressure levels and numbe of swallowings This is obvious from Tabl VII Positive aspiration means cases with th capacity of equilibrating negative middle ea pressures i e group I and II There are grea differences in the aspiration capacit (7 5–70%) the differences probably depend ing on several factors

The state of the middle ear mucosa an thereby the patient selection is probably one o the most important causes of the differences Miller found a statistically significant reduc tion of the tubal function in ears with "dis eased mucosa compared with ears with nor mal mucosa He showed that the tubal func *tion recovers after antibiotic treatment* Onl patients in whom the ears had been dry for a least 2 months were accepted in the present study Testing during upper respiratory tract *infection also affects the results* This stresses the importance of testing only patients with dry ears and without respiratory tract infec tion

The initial test pressure level might be re sponsible for some of the differences in Table VII Physiologically, the Eustachian tube works with low pressure differences of 0–5 cm

(Zollner, 1942, van Dishoeck, 1947, nsen, 1957, Ingelstedt et al , 1963, 1967, t al , 1966, and Elner et al , 1971) How- clinical testing at such low gradients is ult with the closed method Table III vs that the percentage of patent tubes in ses with increasing test pressure levels, cially at positive middle ear pressures ierg (1966) found high flow resistance in Eustachian tube at negative pressures be- -10 cm H_2O in the middle ear, which was ight to be an effect of congestion of the osa This "mucosal factor" was quanti- d by Andreasson et al (1975) Testing at) cm H_2O allows easy recording without unduly effect on the mucosa and therefore ms to be suitable when testing tubal func i In all the other studies higher initial test ssure levels have been used Ingelstedt et (1967) and Rundcrantz (1969) found that ial function varies with posture When the iject was recumbent the volume of the tubal w was only one third of that recorded when test person was sitting In order to get the st tubal function the test must be done with patient sitting This has been done by isberg Holmquist and Ekvall but has not en mentioned in the other investigations Table VII)

In the other part of this investigation Andreasson 1975) a correlation was found etween tubal function and the volume of the ir-filled system but not between tubal func- on and the planimetrically assessed area of ie cell system This means that the volume nd the tubal function probably depend on the xtent of the mucosal damage in the whole ystem—middle ear mastoid air cell system ind Eustachian tube This also suggests the alue of preoperative tubal function testing as vell as volumetric determinations

Clinical recommendations

With the new equipment just described the following test performance is recommended

Test only patients with dry ears and no signs of upper respiratory infection and perform test with the patient sitting

(1) Politzer's test If negative, no further test is needed
(2) Valsalva's manoeuvre
(3) Toynbee's test If positive, point 4 can be excluded
(4) Aspiration-deflation Initial test pressure level ± 10 cm H_2O, 10 swallows at most during 3 min
(5) Volume measuring of the air filled ear spaces (V_m)

This is technically very simple as all instructions are given on the equipment The method does not require any training of the staff The test takes 10–15 min Every case in the present material has been or will be operated on regardless of the results of the test The late results of operation will be published in due course

ZUSAMMENFASSUNG

Es bestehen unterschiedliche Auffassungen uber den Nutzen einer praoperativen Untersuchung der Ohren trompete Die Resultate variieren sehr stark und bisher ist unseres Wissens keine vergleichende Untersuchung von verschiedenen Tests der Tubarfunktion publiziert worden Von 132 Ohren mit zentraler Trommelfellperfora tion von denen 9 traumatisch waren und der Rest auf eine chronische Otitis media zuruckzufuhren war wurde die Tubarfunktion untersucht und zwar mit einer geschlos senen manometrischen Methode und mit einer offenen Methode die das Volumen der Tubarpassagen misst Die Resultate der geschlossenen und offenen Methode stimmten in 96% der Fälle uberein Die offene Methode bringt exaktere Werte wahrend die geschlossene ein facher auszufuhren ist 70% der Gesamtfälle hatten Aspi rationskapazitat d h man konnte den Druck im Mittelohr neutralisieren dies ist besser als bei fruheren Unter suchungen Toynbees Test ist ein guter screening test und wenn er positiv ist liegt eine gute Tubarfunktion vor Ein enger Zusammenhang bestand zwischen der Tubarfunktion und dem Volumen des Zellsystems als ein Effekt des generellen Schleimhautschadens

REFERENCES

Andreasson L 1973 *Kronisk slemhinneotit* Preoperativ bedomning Malmo Allmanna Sjukhus Tryckeri Malmo Sweden

— 1975 Chronic otitis Functional volume of the air filled

ear spaces and Eustachian tube function *Ann Otol Rhinol Laryngol* In press

Andreasson L Ingelstedt S Ivarsson A Jonson B & Tjernstrom Ö 1975 Pressure-dependent variation in volume of mucosal lining of the middle ear *Acta Otolaryngol* (Stockh) *81* 442

van Dishoeck H A E 1947 Resistance measuring of the Eustachian tube and the ostium and isthmus valve mechanism *Acta Otolaryngol* (Stockh) *35* 317

Ekvall L 1970 Eustachian tube function in tympanoplasty *Acta Otolaryngol* (Stockh) Suppl *263* 33

Elner Å Ingelstedt S & Ivarsson A 1971*a* A method for studies of the middle ear mechanics *Acta Otolaryngol* (Stockh) *72* 191

— 1971*b* The normal function of the Eustachian tube A study of 102 cases *Acta Otolaryngol* (Stockh) *72* 320

Flisberg K 1966 Ventilatory studies of the Eustachian tube *Acta Otolaryngol (Stockh) Suppl 219*

Flisberg K Ingelstedt S & Örtegren U 1963 Controlled ear aspiration of air *Acta Otolaryngol* (Stockh) Suppl *182* 35

Holmquist J 1968 The role of the Eustachian tube in myringoplasty *Acta Otolaryngol* (Stockh) *66* 289

Ingelstedt S & Jonson B 1967 Mechanisms of the gas exchange in the normal middle ear Lecture at the Nordic Congress of Otolaryngology Helsingfors 1966 *Acta Otolaryngol* (Stockh) Suppl *224* 452

Ingelstedt S Ivarsson A & Jonson B 1967 Mechanisms of the human middle ear Pressure regulation in aviation and diving A non traumatic method *Acta Otolaryngol (Stockh) Suppl 228*

Ingelstedt S & Örtegren U 1963 Qualitative testing of the Eustachian tube function *Acta Otolaryngol* (Stockh) Suppl *182* 7

Jonson B 1969 A flow regulating valve for use in respiratory physiology *Scand J Clin Lab Invest 24* 127

McKinnon D M 1970 Relationship of pre-operative Eustachian tube function to myringoplasty *Acta Otolaryngol* (Stockh) *69* 100

Miller Jr G F 1965 Eustachian tubal function in normal and diseased ears *Arch Otolaryngol 81* 41

Miller Jr G F & Bilodeau R 1967 Preoperative evaluation of Eustachian tubal function in tympanoplasty *South Med J 60 868*

Niwa H 1972 The study on the measurement of Eustachian tubal function *Jap J Otol Abstracts* 6

Palva T Palva A & Karjä J 1969 Myringoplasty *Ann Otol Rhinol Laryngol 78* 1074

Riu R Flottes L Bouche J & LeDen R 196 physiologie de la trompe d Eustache Applications cliniques et therapeutiques Arnette Paris

Rundcrantz H 1969 Posture and Eustachian tube function *Acta Otolaryngol* (Stockh) *68* 279

Sharp M 1970 The manometric investigation of function with reference to myringoplasty results *Laryngol Otol 84 545*

Siedentop K H 1968 Eustachian tube dynamics s the mastoid air cell system and results with tympanoplasty *Otolaryngol Clin North Am 5* 33

Siedentop K H Tardy M E & Hamilton L R Eustachian tube function *Arch Otolaryngol 88*

Smyth G D L Kerr A G & Goodey R J Current thoughts on combined approach tympanoplasty Part I Indications and preoperative assessment *J Laryngol Otol 85* 205

Thomsen K A 1957 Investigations on the tubal function and measurement of the middle ear pressure in pressure chamber *Acta Otolaryngol* (Stockh) Suppl 269

Zollner F 1942 *Anatomie Physiologie Pathologie Klinik der Ohrtrompete* Springer Berlin

L Andreasson M D
Dept of Otolaryngology
Malmo General Hospital
S 21401 Malmo
Sweden

Acta Otolaryngol 82 11–15, 1976

CLASSICAL CONDITIONING OF THE STAPEDIUS MUSCLE

A Yonovitz

From the University of Texas Health Science Center at Houston Speech and Hearing Institute Houston Texas USA

(Received August 4 1975)

ct A delayed conditioning paradigm was employed condition the stapedius muscle While many previous pts have failed using loud sound as the uncondi oned stimulus (UCS) this paper presents evidence of ccessful conditioning of this muscle when the uncondi *stimulus was a mild electrocutaneous stimulation* the external ear canal Using a weak tone as the con tioned stimulus conditioned responses were readily ob ined in eight of ten subjects after only 30 or fewer tone-pairs Temporal conditioning was also demon A parallel is shown to exist between attempts condition the pupillary and stapedius reflexes

ne question of the conditionability of the tapedius muscle is in an unresolved state itive results, though weak and evanescent, e been reported in some studies with cat man, while negative results have been ally reported in most studies

The methods used by the investigators in hese studies are as different as their results Simmons et al (1959), using a reduced coch-ear microphonic in cat as an indication of the coustic reflex, paired light flashed and loud ounds After a number of pairings, the light ash produced a reduction in the cochlear out ut

Coles (1963) found that when human bjects were warned prior to the onset of small arms firing less temporary threshold shift (TTS) was produced that when they were ot Similarly, Brasher et al (1969) reported when a subject anticipated a pistol shot, a lick substituted for the shot produced an in-ation of middle ear muscle contraction

Various reports have shown the middle ear muscle reflex to anticipate a very loud audi tory sequence (Murray & Reid, 1946, Coles & Knight, 1965, Djupesland, 1965, Bench, 1971) Most recently Bench (1971) has shown anti-cipatory contraction of the stapedius muscle to stimuli of 138 dB re 0 0002 dynes/cm^2 Bates et al (1970) and Brainerd & Beasley (1971) re-ported generally negative results in their at-tempts to condition the acoustic reflex as in dicated by the acoustic impedance bridge Bates et al (1970) states "Results were am-bigous in that conditioned responses ap-peared to occur (unreliably) in some Ss but not in others "

In a somewhat comparable situation, the dilation of the pupil has been reported not to be conditionable when its natural afferent arc, the optic nerve, initiated the dilation (Young, 1965), but to be readily conditionable when the unconditioned stimulus (UCS) was an electric shock (Gerall et al , 1957) For the stapedius, Klockhoff (1961) showed that contraction of this muscle may also be elicited with an elec-trical stimulus He found that electro-cutaneous stimulation of the external ear canal produces a homolateral contraction of the stapedius muscle and furthermore that this contraction is the result of an afferent arc and is not due simply to direct electrical stimula-tion of the muscle

In the present investigation, the electro-cutaneous stimulus was the UCS, and the



Fig 1 The modified probe tip with tinsel wire serving as external e[ar] canal electrode

presence of the reflex was assessed by impedance changes (Djupesland, 1964) Data are presented which suggest that the stapedius muscle can be conditioned in the human It is not yet understood why conditioning does not readily occur with a loud sound as the UCS

METHOD

Subjects

Ten young Naval enlisted men were used

Apparatus

A Madsen Model Z0–70 electro-acoustic impedance bridge was used to indicate any change in the middle ear impedance due to stapedius contraction

Tinsel wire having a cloth like consistency was wrapped around the probe tip of the bridge Fig 1 illustrates the electrode A ground electrode was also attached to the pinna

The conditioned stimulus (CS) was delivered by a Permoflux PDR-600 earphone in an MX-41/AR cushion A Grass Model 11 stimulator provided the DC voltage for the UCS The CS and UCS timing were automatically controlled to the nearest 0 01 sec with stan[d]ard laboratory apparatus

The output of the impedance bridge pr[o]vided a voltage which changed with chang[es] in the acoustic impedance of the ear Th[e] voltage caused a deflection of a recordi[ng] pen and provided an indication of stapedi[us] contraction The deflection was measured [in] mm This and the UCS were permanently r[e]corded with a two-channel Beckmann Recor[d]ing Dynagraph

Procedure

A delayed conditioning paradigm was em[]ployed with the CS terminating as the UC[S] came on The CS consisted of a 1-kHz pu[re] tone, 750 msec in duration (40 msec rise fa[ll] time) at 30 dB Hearing Level (HL) This valu[e] was at least 30 dB down from any indicatio[n] of an acoustically-elicited stapedius contrac[]tion The CS was deliverd to S's L ear Th[e] UCS consisted of a 1-second DC voltage t[o] the R external auditory canal via the probe ti[p] used with the Madsen Impedance Bridge Th[e] voltage was increased, providing that there was no pain to the S, until a near maximum stapedius contraction was observed with the

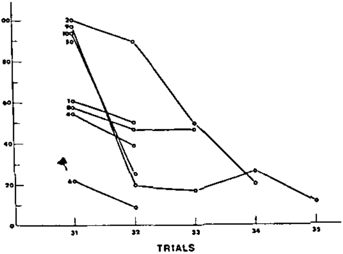


Fig 2 Extinction Data The CR/UCR magnitude is the ratio of the CR s to the initial UCR Each subject conditoned is shown on this graph

... bridge A few CS's were then ...ven to the S as a check against any sensitiza- ... which may have occurred No sensitiza ...on was evident The intertrial interval was a ... 30 sec

Thirty CS UCS pairings were given to each ... at the end of which the CS was delivered ..., until any conditioned responses (CR), ...any, had been extinguished

RESULTS AND DISCUSSION

...'s were observed in eight of ten Ss Fig 2 ...ts the variability among these Ss during ...on Number of CR's varied from 1 to 5 ...efore extinction occurred, indicating (when ... considers that only 30 pairings were pre- ... a fairly strong CR

Two phenomena occurred worth special note

(1) The CR often occurred prior to the UCS during the first 30 trials Continuous recordings were not collected, only occasional samples, during the initial 30 pairings Fig 3 is the 15th trial from S 9 As can be observed, the stapedius muscle begins contraction before the onset of the UCS

(2) Temporal conditioning was occasionally present This was not consistent for all Ss Fig 4 indicates the spontaneous contractions in S 3 of the stapedius muscle during the 20th trial, clearly indicating temporal conditioning

There is an extraordinarily close parallel between conditioning of the pupillary and stapedius muscles Both have a history of con-

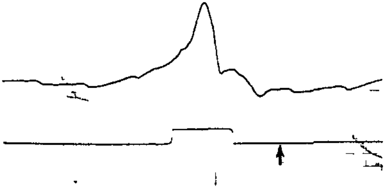


Fig 3 The upper trace represents the impedance change produced by the stapedius muscle The lower trace depicts the UCS The ar row indicates the CS onset The CR occurs prior to UCS onset

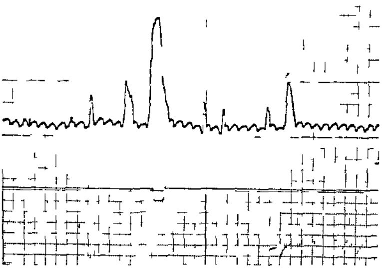


Fig 4 Temporal conditioning in the st dius muscle Again the upper trace i cates impedance change while the lc marks the UCS The intertrial interval a constant 30 sec

trasting reports The present report makes the parallelism more dramatic in that early failures to condition pupillary dilation (Young, 1958) were followed by notable successes in condi tioning when not a visual stimulus, but elec tric shock was used to elicit the pupillary reflex (Gerall et al , 1957) As Kimble (1961) puts it "It seems likely that the emotional ac companiments of the shock may have been important" Both the stapedius muscle con traction and pupil dilating may very well be a part of a generalized UCR to shock or noxious Young (1965) contends The nature of the generalized response in terms of latency and magnitude is modified during the course of conditioning in the expected direction so that comparable changes in the behavior of the pupil probably represent changes occurring in the generalized response It is now important to test this hypothesis in the case of the stapedius muscle by including an index of the generalized response such as the galvanic skin response

ACKNOWLEDGEMENT

Appreciation is extended to the Aud tory Branch of the Naval Submarine Medical Research Laboratory Groton Conn for use of subjects and facil ties

ZUSAMMENFASSUNG

Ein verzogertes bedingtes Paradigma wurde angewandt um den Staped usmuskel empfindlich zu machen Obwohl zahlreiche fruhere Versuche die lauten Ton als u: dingten Reiz gebrauchten nicht gelungen sind b diese Abhandlung Beweise dar daß dieser Muskel erl reich empfindlich gemacht werden kann wenn mar unbedingten Reiz eine geringe elektrische Hautstim tion des außeren Gehorganges anwendet Ein bedin Reflex wurde ohne Schwierigkeit in acht von zehn I viduen nach Verabfolgung von dreiß g oder sogar wen Ton Schock Gruppen ausgelost wenn man einen le Ton als bedingten Reiz gebrauchte Zeitliche Bed ng wurde auch demonstriert Man beweist daß eine P: lele zwischen den Versuchen an bedingten Pupille flexen und Stapediusreflexen besteht

REFERENCES

Bates M A Loeb M A Smith R & Fletcher J 1970 Attempts to condition the acoustic ref *J Audit Res 10* 132

Bench J 1971 Anticipatory elicitation of the mid ear muscle reflex *J Laryngol Otol 85* 1161

Brasher P F Coles R R A Elwood M A & Fer H N 1969 The influence of middle ear muscle a vity on auditory threshold shifts induced by no *J R Army Med Corps 115* 1

Brainerd S H & Beasley D S 1971 A failure to el respondent conditioning of the stapedius reflex *Audit Res 11* 234

Coles R R A 1963 Some considerations concerning effects on hearing of noise of small arms *J R N Med Serv 49* 1

Coles R R A & Knight J J 1965 The problem of no in the Royal Navy and Royal Marines *J Laryn Otol 79* 131

Djupesland G 1964 Middle ear reflexes elicited acoustic and non acoustic stimulation *Acta O laryngol* (Stockh) Suppl *188* 287

— 1965 Electromyography of the tympanic muscles man *Int Audiol 4* 34

Gerall A A Sampson P B & Boslov G L 1957 Cl

ical conditioning of human pupillary dilation *J Exp sychol 54* 467
ble G A 1961 Hilgard & Marquis *Conditioning and earning* 2nd ed New York Appleton Century rofts p 51
khoff I 1961 Middle ear muscle reflexes in man *Acta Otolaryngol* (Stockh) Suppl *164*
ray N E & Reid G 1946 Temporary deafness due o gun fire *J Laryngol Otol 61* 609
mons F B Galambos R & Rupert A 1959 Conditioned response of middle ear muscles *Am J Physiol 197* 537
ing F A 1965 Classical conditioning of autonomic functions In *Classical conditioning* A Symposium (ed W F Prokasy) Appleton Century-Crofts New York 1965
— 1958 Studies of pupillary conditioning *J Exp Psychol 55* 97

A Yonovit Ph D
University of Texas
Health Science Center at Houston
Speech and Hearing Institute
1343 Moursund
Houston Texas 77025 USA

Acta Otolaryngol 82 16–25, 1976

HOLOGRAPHIC VIBRATION ANALYSIS OF THE OSSICULAR CHAIN

T Gundersen[1] and K Høgmoen[2]

From the Departments of [1]Otolaryngology and [2]Physics The Norwegian Institute of Technology University of Trondheim Trondheim Norway

(Received August 28 1975)

Abstract The mechanics of movement of the ossicular chain has been investigated on human temporal bone preparations by means of time average holography The malleus and incus moved like a lever around an axis which position was somewhat dependent upon frequency Simulation of contraction of the muscles of the middle ear gave as result a change in the pattern of movement of the ossicular chain The position of the rotatory axis was changed and the amplitudes of vibration were reduced This should be a protecting mechanism

As a result of developments in ear surgery there has been an increase in interest in the significance of the middle ear for the transmission of sound from the tympanic membrane to the oval window Exact knowledge of this part of the mechanical system of the ear is necessary both for predicting the outcome of defects in the system and for deciding how to treat such defects

The amplitudes of vibration in the ossicular chain are very small under physiological conditions and the amount of energy which is necessary to initiate the movement is also small This and the anatomy of the middle ear make it difficult to measure the vibrations No apparatus has yet been developed that makes it feasible to investigate the mechanics of movement of the ossicular chain in living humans In our experiments we used temporal bones from human cadavers

The holographic method is well suited for measurements on temporal bone preparations It is a non contact method it shows vibration amplitude distribution on the wl object, and its sensitivity range makes it sible to measure the amplitudes in the cular chain at physiological sound pres levels and relatively high frequencies

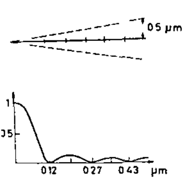
The patterns of movement were essenti the same for all preparations but the vibra sensitivity against the input sound pres showed considerable variation from preparation to another It has therefore b necessary to give this work a descrip character

THE EXPERIMENTS

Preparation of the object to be measured

The experiments were carried out on fr temporal bones which by macroscopic ex ination did not show any pathological chan The preparation of the temporal bones is scribed earlier by Gundersen (1971 197 but no probe microphone was connected the round window in the investigations d scribed here Gundersen (1971) also gives detailed valuation of factors influencing t validity of data recorded from such tempor bone preparations

The temporal bones were removed fro 6 to 48 hours after death from cadave



1 (A) Amplitude along a vibrating bar Positions re the amplitude corresponds to max and min ne J_0^2 function are marked (B) Light intensity distri on of the reconstructed picture of the bar by time ~ge holography

oled in the meantime to +4°C The bones prepared immediately after removal, d the measurements were performed the or the following day If the experiments ld not be performed immediately after ation, the bones were stored wet in an tight plastic bag, and cooled to +4°C scles and connective tissue were removed the surface of the bone during prepara The skin in the auditory meatus was re-ed From the digastric fossa a hole was ed through the innermost part of the mea A probe with an inner diameter of 3 3 mm a length of 22 mm was fixed to the meatus that the probe was at a distance of 2 to 3 from the eardrum Previously it has been own that the age of the patient at the time death has no influence on investigations the mechanics of ossicular movement The interval between death and measurement is also without importance, within certain limits (Elpern & Andersen, 1965; Gundersen, 1971)

Apparatus

Holographic vibration analysis is well suited to this kind of examination because vibratory patterns of the object as a whole are recorded, giving a reconstructed picture of the object superimposed on a pattern of dark and bright lines These "iso amplitude contour lines" give the vibration amplitudes of the object during the recording of the hologram Tonndorf & Khanna (1972) have used this method in investigating the movements of the drum

Contrary to the use of an electromagnetic pick-up, the method does not exert any significant extra load on the object The only extra load on the object was a thin layer of aluminium powder (particle size about 1 μm) as described by Khanna (1970), applied to give the object a diffusely reflecting surface The weight of the aluminium powder was about 0 03 mg/cm², uniformly distributed over the surface of the object

By time average holography (Powell & Stetson, 1965) the relationship between the reconstructed intensity and vibration amplitude is given by a squared zero order Bessel function (J_0^2), which is drawn in Fig 1 Fig 1 also shows a stiff vibrating bar in order to clarify the relationship between light intensity and

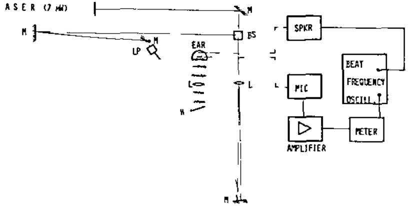


Fig 2 Experimental arrangement *M* Mirror *BS* Beamsplitter *L* Lens/microscope objective *LP* Lens pinhole spatial filter *H* Hologram *SPKR* Loudspeaker, *MIC* Microphone

vibration amplitude The first zero of the J_0^2-function corresponds to an amplitude of 0 12 μm, which gives the first dark fringe in the reconstructed picture One will get a dark fringe in the reconstruction wherever the amplitude has increased by about λ/4, where λ is the wavelength of the employed laser beam (He–Ne laser λ=0 6328 μm) Areas of the object that are at rest will be reconstructed with maximum intensity and will give the brightest fringes on the picture

Fig 2 shows the experimental arrangement It is an ordinary holographic set-up, where the laser beam is split into an object wave and a reference wave Because the ossicular chain is a relatively small object, it was viewed through a microscope We placed the hologram plate between the objective and the eye-piece We could then vary the magnification in the reconstruction process by substitution of different eye-pieces The input signal was passed from the loudspeaker via a rubber tube to the auditory meatus The probe microphone was used to control and regulate the sound pressure on the eardrum A stable holder for the ear was constructed and, in addition, the usual precautions required to fulfil the strict stability requirements of holography were observed There was no problem arising from background noise during the experiments

Method

So far, 32 temporal bone preparations have been used for this investigation

About one hour was required for the alignment procedure During alignment and before recording the holograms we moistened the preparation, in order to keep it from drying We also kept a relative humidity of about 60% in the laboratory during the experiments

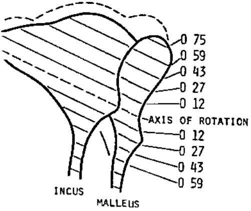
The recording of a series of holograms using 30 combinations of frequencies and sound pressures took from ½–1 hour Control recordings were made at the start and end of each series in order to check that nothing had changed during the experiments The holograms were recorded on Agfa-Scientia 10E plates, and the exposure time was ½ seco[nd] Recordings were made with sound press[ure] levels (SPL) from 85–140 dB re 2×10^{-5}N/[m²] and with frequencies from 100–10000 [Hz] Most of the recordings were made with f[re]quencies below 2000 Hz, and with SPL fr[om] 100–125 dB, because these produced vibrat[ion] amplitudes which were well suited for [the] sensitivity range of ordinary time aver[age] holography

RESULTS

In this paper the movements of malleus a[nd] incus are described We also simulate the [ef]fect of the middle ear muscles on the patte[rn] of vibration The behaviour of the sta[pes] footplate will be considered in a forthcom[ing] paper

Malleus and incus

In order to visualize malleus and incus fr[om] the medial side, a part of the semicircu[lar] canals and vestibulum had to be remov[ed] and thereby the liquid load exerted by an [in]tact cochlea was taken away Without remo[v]ing these parts of the cochlea it was impossi[ble] to see both the tip of the manubrium bo[th] of the malleus and the long crus of the inc[us] To investigate the effect of this encroachme[nt] a series of recordings at different frequenc[ies] and sound pressures was made from som[e] preparations with intact cochlea Then, on t[he] same preparations, a hole was made to th[e] superior semicircular canal, the perilymp[h] was drained out, and the series of recording[s] was repeated This was done on 3 prepara[tions] and judging by the results we could no[t] say that the mode of vibration was altere[d] by the draining out of the perilymph Th[e] vibratory pattern was the same as before but the amplitudes of vibration became somewhat larger after the perilymph was drained out This is in agreement with Gundersen's (1971) results from measurements with electromagnetic pick-up





3 Hologram reconstructions of malleus and incus 1090 Hz 124 dB *The explanatory drawing* shows the pos tion of the ax s of rotation and the ampl tude at the d fferent fringes given n μm

The vibratory pattern of malleus and incus frequency 1 090 Hz and 124 dB SPL is given Fig 3 The explanatory drawing shows e interpretation of the pictures The axis of tation is in the middle of the brightest fringe rom the position of the fringes and because ley represent straight lines across malleus id incus one can conclude that malleus and cus rotate like one stiff body around the xis Large non physiological sound pressures re needed in order to produce movement i the joint between malleus and incus This ill show up as a discontinuity or deflection f the straight fringes by transition from mal us to incus

The axis of rotation does not pass through he tip of the short process of the incus but omewhat further down on the incus body On he malleus the axis passes through the mal ‿us neck

Fig 4 gives a series of photographs from iologram reconstructions of malleus and in us at different frequencies and sound pres ures Accurate measurements on the recon tructed pictures show that the axis of rotation emains in the same position when increasing he sound pressure at a fixed frequency On he other hand the axis is shifted when the requency is changed which can be seen by carefully comparing recordings made at differ ent frequencies It is most easily seen from the pictures at 196 Hz–124 dB and 930 Hz–124 dB

Earlier measurements have shown that the *ossicular chain* has a *point of resonance usu* ally at a frequency between 800–900 Hz (Gun dersen 1971) When the frequency is increased to this point of resonance the axis moves away from the malleus head and further down on the malleus neck When the frequency is increased above the point of resonance the axis moves away from the malleus head and further down on the malleus neck When the frequency is increased above the point of resonance the axis seems to shift towards the malleus head again but the determination of the axis position is here more uncertain This is because the amplitude of vibration de creases rapidly above the point of resonance and consequently accurate determination of the axis position requires larger sound pres sure

The shifting of the axis position makes the ratio of the ossicular chain smaller at the point of resonance and larger by frequencies below and above this point

Several experiments were performed in order to detect a piston like movement in

vibration amplitude The first zero of the J_0^2 function corresponds to an amplitude of 0 12 μm, which gives the first dark fringe in the reconstructed picture One will get a dark fringe in the reconstruction wherever the amplitude has increased by about $\lambda/4$, where λ is the wavelength of the employed laser beam (He–Ne laser $\lambda=0\ 6328\ \mu$m) Areas of the object that are at rest will be reconstructed with maximum intensity and will give the brightest fringes on the picture

Fig 2 shows the experimental arrangement It is an ordinary holographic set up, where the laser beam is split into an object wave and a reference wave Because the ossicular chain is a relatively small object, it was viewed through a microscope We placed the hologram plate between the objective and the eye piece We could then vary the magnification in the reconstruction process by substitution of different eye pieces The input signal was passed from the loudspeaker via a rubber tube to the auditory meatus The probe microphone was used to control and regulate the sound pressure on the eardrum A stable holder for the ear was constructed and, in addition, the usual precautions required to fulfil the strict stability requirements of holography were observed There was no problem arising from background noise during the experiments

Method

So far, 32 temporal bone preparations have been used for this investigation

About one hour was required for the alignment procedure During alignment and before recording the holograms we moistened the preparation, in order to keep it from drying We also kept a relative humidity of about 60% in the laboratory during the experiments

The recording of a series of holograms using 30 combinations of frequencies and sound pressures took from ½–1 hour Control recordings were made at the start and end of each series in order to check that nothing had changed during the experiments The holograms were recorded on Agfa Scientia 10E plates, and the exposure time was ½ secon[d] Recordings were made with sound pressu[re] levels (SPL) from 85–140 dB re 2×10^{-5} N/m[²] and with frequencies from 100–10000 H[z] Most of the recordings were made with fr[e]quencies below 2000 Hz, and with SPL fro[m] 100–125 dB, because these produced vibrati[on] amplitudes which were well suited for t[he] sensitivity range of ordinary time avera[ge] holography

RESULTS

In this paper the movements of malleus a[nd] incus are described We also simulate the e[f]fect of the middle ear muscles on the patter[n] of vibration The behaviour of the stap[es] footplate will be considered in a forthcomi[ng] paper

Malleus and incus

In order to visualize malleus and incus fro[m] the medial side, a part of the semicircul[ar] canals and vestibulum had to be remove[d] and thereby the liquid load exerted by an i[n]tact cochlea was taken away Without remo[v]ing these parts of the cochlea it was impossib[le] to see both the tip of the manubrium bo[th] of the malleus and the long crus of the incu[s] To investigate the effect of this encroachmen[t] a series of recordings at different frequenci[es] and sound pressures was made from so[me] preparations with intact cochlea Then, on t[he] same preparations, a hole was made to t[he] superior semicircular canal, the perilymp[h] was drained out, and the series of recordings was repeated This was done on 3 prepara[ra]tions and judging by the results we could no[t] say that the mode of vibration was altere[d] by the draining out of the perilymph The vibratory pattern was the same as before but the amplitudes of vibration became somewhat larger after the perilymph was drained out This is in agreement with Gundersen's (1971) results from measurements with electromagnetic pick up

lition to the rotating movement This kind movement has been mentioned by Helm tz (1868) and many later investigators ombination of a rotating movement and a ton like movement will give the same result a pure rotating movement with shifting of axis position We have observed a small quency-dependent shifting of the axis Be ise the axis position is not altered when sound pressure is increased at a fixed fre ency there must be a linear relationship tween sound pressure possible piston like ovement and rotating movement

fluence of tensor tympani and apedius muscles

is difficult to investigate the influence of the mpanic muscles by experiments on prepara ons of human temporal bones Among hers E B Neergaard et al (1963) simulated e influence by applying forces to musculus nsor tympani and musculus stapedius

In our experiments the external force was pplied both by traction perpendicularly from gmen tympani on the tendon of tensor tym ani and by pulling the muscle in its natural irection of contraction The traction force n the tendon of musculus stapedius was pplied from tegmen tympani and perpendic larly to the tendon The preparation of the mporal bone included removal of the teg ien tympani allowing access to fasten small ooks around the muscles In order to apply force simulating the natural contraction of ensor tympani the muscle was dissected ree and a thread was fastened to it making t possible to apply the force in the direction f natural contraction We do not know the nagnitude of the force that musculus tensor ympani and musculus stapedius can exert n man In cats Wever et al (1955) found that he average maximum force exerted by ten or tympani was 4 4 g and by stapedius 1 6 g In rabbits Wersall (1958) found the iverage maximum strength of stapedius to be 3 6? g Teig (1973) reports the average force in musculus stapedius of cats by response to a single stimulus to be 3 70 g and to tetanic stimulation to be 13 90 g For tensor tympani he gives 13 58 g as single twitch response and 54 25 g during tetanic contractions In rabbits he reports the following figures in stapedius 7 14 g twitch responses and 5 38 g during tetanic contractions and in tensor tympani 16 0 g twitch and 3? 34 g during tetanic contractions

Our holograms were recorded at frequencies from 300–1 000 Hz and SPL from 114–134 dB We applied forces from 10–50 g to tensor tympani and from 1–10 g to stapedius We also made recordings with combinations of these forces applied to both tensor tympani and musculus stapedius During our series of experiments there were so many possible combinations of muscle force frequency and sound pressure that it was not possible to perform a thorough investigation of the influence of the tympanic muscles in this way For example we have not investigated the dependence on frequency We had to choose some parameters and study the effect of variations in them during the series of measurements In addition it was necessary to make some control recordings before and after each series of measurements in order to check that nothing had changed during the measurements The control recordings showed that when the traction on the muscles ceased malleus and incus again vibrated as before the force was applied

Traction applied to tensor tympani causes movement in the joint between malleus and incus This shows up on the hologram reconstruction by a discontinuity of the interference fringes by transition from malleus to incus as can be seen from Figs 5B C and 6A There are more fringes on the malleus head than there are on the incus which implies that the malleus has a larger amplitude of vibration than the incus Malleus and incus seem to have an axis of rotation whose position is somewhat closer to the malleus head than it is without traction to tensor tympani see explanatory drawing in Fig 5

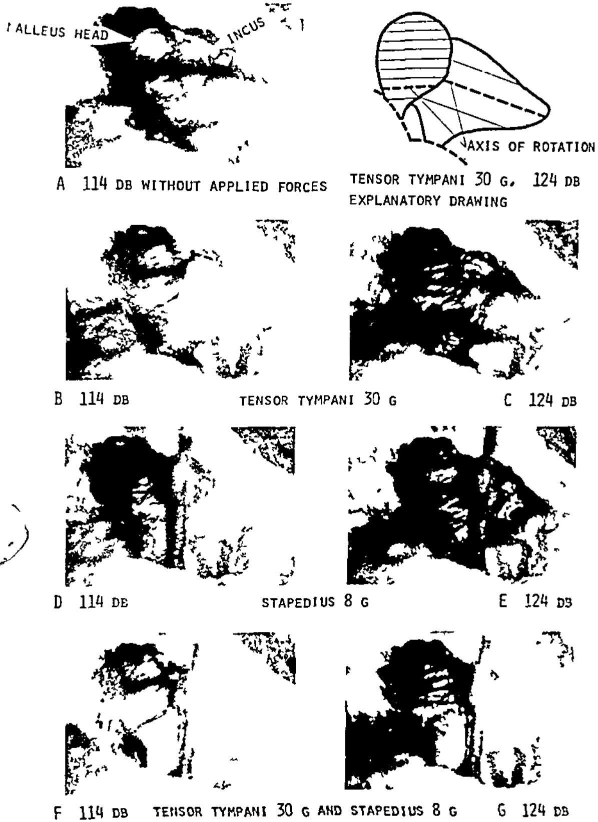
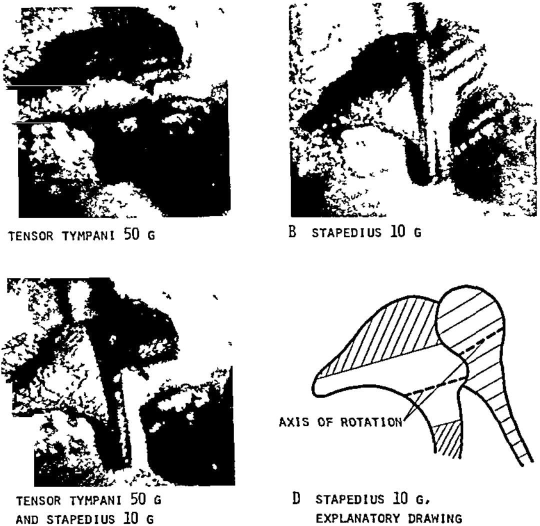


Fig 5 Hologram reconstructions of malleus and incus at 600 Hz 114 and 124 dB with different forces applied to the muscles



g 6 Malleus and incus at 1000 Hz and 124 dB with
orces applied to the muscles

The explanation of the reduced transfer of novement from malleus to incus when pulling n tensor tympani, might be that the pull auses some distraction between the joint aces, and thereby leads to reduced frictional orces in the joint When it contracts, muscu us tensor tympani pulls the malleus forward nd medial in the epitympanic recess The vhole ossicular chain will be pulled forward nd inward the elasticity in ligamentum an ıulare will then result in a counterforce The ounterforce will make it more difficult for he incus and stapes to follow the vibrating novement of the malleus

The force exerted on tensor tympani also results in attenuation of the vibration ampli tudes of the malleus Fig 7 gives the ampli tude at the top of the malleus head (fully drawn line) as function of force applied to tensor tympani at 940 Hz and 114–124 dB SPL The measurements were made with 0, 10, 20 and 30 g applied force Here, an increase in force by 10 g caused a reduction of the ampli tudes on the malleus of about 3 dB

Vibration amplitudes measured at the top of the incus as a function of force applied to ten sor tympani are also shown in Fig 7 (dotted line) The amplitudes here are smaller than

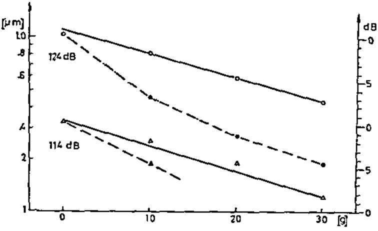


Fig 7 Amplitudes of vibration a[illegible] a funct on of force appl ed to ten[illegible] tympani at 940 Hz 114 dB (lower curves) and 124 dB (upper curves The measurements are from the malleus head (——) and from the top of incus ( ) they are evalua[illegible] from hologram reconstructions

they are at the malleus This is a result of the movement in the joint between malleus and incus At 114 dB SPL the amplitude of the incus became too small to be measured by 20 and 30 g applied force (the first dark fringe in the reconstructed picture occurs at 0 12 μm amplitude)

Traction force exerted on musculus stapedius attenuates the movements much more than the same force applied to tensor tympani As an example one series of measurements at 940 Hz and 124 dB showed that 2 g force applied to stapedius reduced the amplitude of incus by the same amount as 20 g applied to tensor tympani It was difficult in our experiments to apply forces smaller than 1 g As a result all vibration amplitudes became very small when applying force to musculus stapedius thereby giving more uncertainty concerning the mode of vibration The pull on the stapedius results in a discontinuity of the axis of rotation The incus rotates around an axis whose position is somewhat lower down on the body This means that the ratio of the ossicular chain becomes smaller On the malleus the axis still goes through the malleus neck (see Figs 5D E and 6B D) Here too it can be seen that there is movement in the joint between malleus and incus

Figs 5 and 6 illustrate the combined action of musculus tensor tympani and musculus stapedius When applying forces to both muscles at the same time the effect becom[illegible] greater than when applying force to each [illegible] the muscles separately The effect does n[illegible] seem to be a pure additive effect

CONCLUDING REMARKS

Our experiments on human temporal bon[illegible] showed

The ossicular chain moves like a lev[illegible] around an axis The axis position is slight[illegible] dependent upon frequency The ratio of th[illegible] ossicular chain is smaller at the point of re[illegible]onance and larger at frequencies below a[illegible] above this point

Traction in the tympanic muscles atte[illegible]uates the vibration amplitudes and reduc[illegible] the transmission of amplitude from malle[illegible] to incus This is a result of movement in th[illegible] joint between malleus and incus This mu[illegible] be considered a protecting mechanism

Head injuries and large acustotraumas ofte[illegible] lead to discontinuity of the ossicular cha[illegible] by breaking the connection between malleu[illegible] and incus A possible explanation might b[illegible] a combination of head injury or large soun[illegible] pressures and a sudden contraction of tenso[illegible] tympani To reduce the transmission of am[illegible]plitude one needs to apply a much smalle[illegible] force on the stapes muscle than on the tenso[illegible] tympani *Contraction in the muscles of the* middle ear alters the pattern of movement of the ossicular chain Malleus and incus change

s of rotation thus leading to a change in the io of the ossicular chain Previous investi ions by Gundersen (1971) have shown that mechanics of movement of the ossicular in influences the sensitivity of the ear ntraction of the tympanic muscles alters ratio of the ossicular chain This might ine the ear for better recording at certain quencies

ACKNOWLEDGEMENT

ateful acknowledgement is made to O J Løkberg for dance during this work and for valuable suggestions

ZUSAMMENFASSUNG

Bewegungsmechanik der Gehorknochelchen wurde i Zeitmittel Holographie an Praparaten von Felsen inen untersucht Der Hammer und der Amboß beweg sich wie ein Hebel um eine Drehachse Die Lage eser Drehachse war etwas frequenzabhangig Simula on der Kontraktion der Mittelohrmuskeln gab als Re ltat eine Anderung des Bewegungsmusters Die Dreh hse wurde verschoben und damit wurde das Uber tzungsverhaltnis der Gehorknochelchenkette geändert ie Schwingungsamplituden wurden reduziert und dies llte ein Schutzmechanismus sein

REFERENCES

Elpern B S & Andersen H C 1965 Experimental studies on sound transmission in the human ear *Acta Otolaryngol* (Stockh) *61* 403

Gundersen T 1971 *Prostheses in the Ossicular Chain* University Park Press Baltimore

— 1972 Prostheses in the ossicular chain *Arch Otolaryngol 61* 416

von Helmholtz H 1868 Uber die Mechanik der Gehorknochelchen und des Trommelfells *Pflugers Arch I* 1

Khanna S M 1970 *A Holographic Study of Tympanic Membrane Vibrations in Cats* Ph D thesis City University of New York University Microfilms Ann Arbor Mich

Neergaard E B et al 1963 Experimental studies on sound transmission in the human ear *Acta Otolaryngol* (Stockh) Suppl *188* 280

Powell R L & Stetson K A 1965 Interferometric vibration analysis by wavefront reconstruction *J Opt Soc Am 55* 1593

Teig E 1973 *Experimental Studies on the Middle Ear Muscles* Universitetsforlaget Oslo

Tonndorf J & Khanna S M 1972 Tympanic membrane vibrations in human cadaver ears studied by time averaged holography *J Acoust Soc Am 52* 1221

Wersall R 1958 The tympanic muscles and their reflexes *Acta Otolaryngol* (Stockh) Suppl *139* 1

Wever E G et al 1955 The maximum strength of the tympanic muscles *Ann Otol Rhinol Laryngol 64* 383

T Gundersen M D
Dept of Otolaryngology
University of Trondheim
N 7000 Trondheim
Norway

Acta Otolaryngol 82 26–32, 1976

RADIOIMMUNOASSAY OF IgE IN MIDDLE EAR EFFUSIONS

G Mogi, S Maeda, T Yoshida and N Watanabe

From the Department of Otolaryngology Yamaguchi University School of Medicine Ube Yamaguchi ken Japan

(Received September 29 1975)

Abstract Fifty six sample pairs of middle ear effusions and sera obtained from patients with otitis media with effusion were investigated for the IgE antibody activity against mites using radioallergosorbent test (RAST) which was recently developed as an *in vitro* allergy test The IgE concentrations were also determined by radioimmunosorbent test (RIST) Results showed that the IgE antibody activity against this allergen was found in 5 middle ear effusions and 3 sera Four of 5 patients with the positive RAST test had nasal allergy or signs suggesting allergy There were two middle ear effusions which seemed to be an allergic fluid However findings in the present study do not suggest that middle ear effusions result from the direct allergic reaction induced by mites in the mucous membrane lining the middle ear cavity

Although otitis media with effusion is a common disease constituting a major cause of hearing loss, the etiology and pathogenesis of this disorder is still a controversial topic Many authors (Jordan, 1949, Derlacki, 1952, Lecks, 1961, McGovern, 1967) have regarded allergy as one of the major etiologic factors contributing to otitis media with effusion However, the opinion that allergy does not play any significant role in the formation of middle ear effusion has been put forward by a number of authors (Suehs, 1952, Senturia et al , 1958, Boor, 1962, Sirala 1964, Lim & Birck, 1971)

Since IgE contains reaginic antibodies which are responsible for the Prausnitz Kustner reaction, Mogi et al (1974) measured the IgE concentration in middle ear effusions and corresponding sera Their results suggested that IgE in middle ear effusions, rather than being a local product, may be derived from the serum, and also that the fluid is not an atopic allergic effusion However, their study did not include an investigation of the specific antibody activity of the IgE present in the effusion against certain allergens

The radioallergosorbent test (RAST) (Wide et al , 1967) has come to the fore as a new form of the *in vitro* allergy test and the great value of the RAST for determination of IgE antibodies against many kinds of allergen has been demonstrated by Johansson et al (1972), Wide (1973) and Okuda & Usami (1974) Most of allergens causing respiratory tract allergy in Japan are house-dust and mites (Okuda, 1974 Miyamoto et al , 1974) Moreover, it was demonstrated in RAST studies on atopic allergy in Japanese subjects, that the mite is a major allergenic component of Japanese house-dust (Miyamoto et al , 1974) In order to determine whether or not mites are an antigenic factor causing direct allergic reactions in middle ear cavities, in this study the IgE antibody activity against mites, as well as IgE concentration in middle ear effusions and sera were determined by use of the RAST and radioimmunosorbent test (RIST) (Johansson et al , 1968)

MATERIAL AND METHODS

Fifty six specimens of middle ear effusion were aspirated from 56 patients diagnosed as

This work was supported by grant (number 848193) from the Ministry of Education Japan

I *IgE concentrations and RAST scores of middle ear effusions and sera in the serous-* *category*

Age	Sex	IgE concentration Effusion (U/ml)	Serum (U/ml)	RAST score Effusion	Serum
72	♀	10	20	0	0
61	♂	10	<10	0	0
73	♂	25	25	0	0
77	♀	230	1 350	0	0
61	♀	–*	–	0	0
60	♂	<10	<10	0	0
78	♂	<10	<10	0	0
61	♂	<10	<10	0	0
83	♀	10	10	0	0
51	♂	–	–	0	0
56	♀	–	–	0	0
46	♀	<10	<10	0	0
32	♂	<10	<10	0	0
62	♂	<10	<10	0	0
46	♂	23	10	0	0
43	♂	930	630	0	0
46	♂	<10	<10	0	0
66	♀	1 720	450	0	0
59	♀	10	92	0	0
34	♂	–	–	0	0
50	♀	10	<10	0	0
45	♂	–	–	0	0
53	♂	840	940	0	0
59	♂	<10	35	0	0
8	♂	13	36	0	0

*Not measured

ıving otitis media with effusion The age and of these patients are described in Tables I, and III Specimens were categorized as of ı ɹs or mucoid type by Senturia's classifi tion (Senturia, 1970) The specimens were broken down into acute and chronic types the criterion as described in a previous (Mogi et al , 1973) Thirty nine serous ens were obtained from 25 acute and 14 cases, 17 mucoid specimens were 11 acute and 6 chronic cases A blood ' for serum was collected from each pa at the same time that the effusion was ' Effusion and serum samples were ɹwn off quantitatively, using a microselect pipette [1]

ay Adams Division of Becton Dickenson & Co ppany N J USA
a Diagnostics AB Uppsala Sweden

IgE antibody activity against dermatophagoides farinae

The RAST was performed to investigate the antibody activity of IgE in middle ear effusions and sera against this antigen using Phadebas® RAST kit [2] The RAST is based on the anti globulin principle of Coombs et al (1953) Al though the principle and detail procedure of the RAST are described elsewhere (Johansson et al , 1971), Fig 1 illustrates a schematic ex planation of this principle Duplicate assays were performed on each sample Briefly, 50 μl of the unknown middle ear effusion or serum sample was incubated with a filter paper disc coated with the extract of *dermatophagoides farinae* in a polystyrene tube for 3 hours at room temperature After washing 3 times with saline, 50 μl of the anti IgE ^{125}I solution was added to paper disc and agitated overnight at room temperature The paper disc was washed

Acta Otolaryngol 82 26–32, 1976

RADIOIMMUNOASSAY OF IgE IN MIDDLE EAR EFFUSIONS

G Mogi, S Maeda, T Yoshida and N Watanabe

From the Department of Otolaryngology Yamaguchi University School of Medicine Ube Yamaguchi ken Japan

(Received September 29 1975)

Abstract Fifty six sample pairs of middle ear effusions and sera obtained from patients with otitis media with effusion were investigated for the IgE antibody activity against mites using radioallergosorbent test (RAST) which was recently developed as an *in vitro* allergy test The IgE concentrations were also determined by radioimmunosorbent test (RIST) Results showed that the IgE antibody activity against this allergen was found in 5 middle ear effusions and 3 sera Four of 5 patients with the positive RAST test had nasal allergy or signs suggesting allergy There were two middle ear effusions which seemed to be an allergic fluid However findings in the present study do not suggest that middle ear effusions result from the direct allergic reaction induced by mites in the mucous membrane lining the middle ear cavity

otitis media with effusion is a common disease constituting a major cause of hearing loss the etiology and pathogenesis of this disorder is still a controversial topic Many authors (Jordan, 1949, Derlacki, 1952, Lecks, 1961, McGovern, 1967) have regarded allergy as one of the major etiologic factors contributing to otitis media with effusion However, the opinion that allergy does not play any significant role in the formation of middle ear effusion has been put forward by a number of authors (Suehs, 1952, Senturia et al, 1958, Boor, 1962, Sirala 1964, Lim & Birck, 1971)

Since IgE contains reaginic antibodies which are responsible for the Prausnitz Kustner reaction, Mogi et al (1974) measured the IgE concentration in middle ear effusions and corresponding sera Their results suggested th IgE in middle ear effusions, rather than bei a local product, may be derived from the s rum, and also that the fluid is not an atopic lergic effusion However, their study did n include an investigation of the specific an body activity of the IgE present in the effusi against certain allergens

The radioallergosorbent test (RAST) (W et al, 1967) has come to the fore as a new for of the *in vitro* allergy test and the great value the RAST for determination of IgE antibodi against many kinds of allergen has be demonstrated by Johansson et al (1972), Wi (1973) and Okuda & Usami (1974) Most allergens causing respiratory tract allergy Japan are house-dust and mites (Okuda, 19 Miyamoto et al, 1974) Moreover, it was de onstrated in RAST studies on atopic aller in Japanese subjects, that the mite is a ma allergenic component of Japanese house-du (Miyamoto et al, 1974) In order to determi whether or not mites are an antigenic fact causing direct allergic reactions in middle e cavities, in this study the IgE antibody activ ty against mites, as well as IgE concentratio in middle ear effusions and sera were dete mined by use of the RAST and radioimmuno sorbent test (RIST) (Johansson et al, 196

MATERIAL AND METHODS

Fifty six specimens of middle ear effusio were aspirated from 56 patients diagnosed a

This work was supported by grant (number 848193) from the Ministry of Education Japan

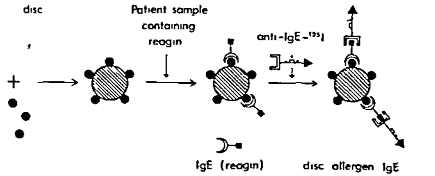
III *IgE concentrations and RAST scores of middle ear effusions and sera in the mucoid* ...ory

Age	Sex	IgE concentration Effusion (U/ml)	IgE concentration Serum (U/ml)	RAST score Effusion	RAST score Serum
type					
7	♂	840	200	1	0
54	♀	400	250	0	0
24	♀	48	10	0	0
8	♂	17	360	0	0
47	♂	<10	<10	0	0
32	♂	18	190	0	0
57	♀	10	10	0	0
54	♀	66	45	0	0
24	♀	165	270	0	0
35	♂	–*	–	0	0
66	♂	85	1 200	0	0
nic type					
58	♀	10	10	0	0
45	♂	10	10	0	0
65	♂	580	630	2	2
73	♀	–	–	1	2
43	♀	–	–	0	0
72	♀	90	130	0	0

ot measured

ear effusions were found to possess ody activity in the IgE The IgE concentrons in the effusion or serum in cases with ositive RAST score were over 500 U/ml wever, in one of these cases, it was impos to test the IgE concentration because of small amount of aspirated effusion

he IgE antibody activity against *dermato‑oides farinae* was present in 5 of 56 (8 9%) middle ear effusions Four of these 5 effusions belonged to the chronic type and 3 of 5 effusions were included in the mucoid category In the corresponding sera of these 5 effusions, 3 were found to have the IgE antibody activity Upon reviewing the history and clinical findings of patients with a positive RAST test, 4 of 5 patients had nasal allergy or signs suggesting allergy



1 The principle of the radioallergosorbent test ST) An excess of allergen is coupled to a CNBr vated paper disc which reacts with the specific IgE he patient sample After washing away nonspecific radioactively labeled anti IgE antibodies are allowed to react with any IgE directed to the allergens on the paper disc The radioactivity of the complex is measured in a gamma-counter The amount of radioactivity is related to the concentration of the specific IgE antibodies in the sample

DISCUSSION

Recent studies (Johansson, 1969, Donovan et al, 1970, Tse et al, 1970, Ishizaka & Newcomb, 1970, Fourcard et al, 1971, Yunginger & Gleich, 1971, Smith et al, 1972, Dahlstrom et al, 1973) have demonstrated an elevated IgE level in sera, and secretions in various atopic allergic disorders Although the determination of IgE level in body fluid is of benefit for diagnosing atopic allergic diseases, another *in vitro* test was recently developed for the diagnosis of allergy by Wide et al (1967) This technique is a quantitative assay for reaginic antibodies, called the radioallergosorbent test (RAST) Recent studies (Johansson et al, 1972, Wide, 1973, Okuda et al, 1974, Torsten et al, 1974) demonstrated that the RAST is very useful for *in vitro* diagnosis of atopic allergy on a routine basis Okuda et al (1974) reported that a positive RAST test was found in 44 of 53 (83 0%) patients with allergic rhinitis due to mites Torsten et al (1974) compared the results of the RAST test with those of skin and provocation tests in children with asthma and hay fever and found that a positive RAST test was combined with a positive prick test in 196 of 235 (83 4%) and with a positive provocation test in 163 of 182 (90%) tests

As there are so many allergenic substances, specific antibody activities of IgE against many different allergens should be tested for However, the amount of aspirated middle ear effusion in most cases in this study was not sufficient to allow testing against two or more allergens In this investigation the mite (*dermatophagoides farinae*) was adopted as an allergen, as mites are a major allergen causing respiratory tract allergy in Japan Results of the present study showed that the IgE antibody activity against mites was present in 5 of 56 (8 9%) middle ear effusions This incidence was nearly identical with that obtained in a study by Miyamoto et al (1974) where 6 of 48 (12 5%) sera from healthy Japanese subjects were found by RAST to have specific antibody activity of IgE against house-dust or mites Results of immunohistologic and immunochemical studies (Donovan et al, 1970 Tada & Ishizaka, 1970) suggested that IgE in secretions is produced locally, which may contribute to the inducing of atopic allergic reactions The present study revealed 2 patients in whom the reaginic antibodies against mites reside only in the middle ear effusion but not in the serum It has been demonstrated that either serous or mucoid middle ear effusion is at least partially a local product (Mogi et al, 1974 *a* and *b*, Ogra et al, 1974) Therefore, these effusions might be an allergic fluid However, this supposition is somewhat uncertain as the RAST scores of these effusions were of the lowest grading

The assumption of a direct causative factor of allergy in otitis media with effusion probably derives from the anatomy where the mucous membrane of the middle ear cavity and Eustachian tube continues to the nasal cavity, paranasal sinuses and nasopharynx The entire mucosa of the upper respiratory tract may respond in a manner similar to specific allergic stimuli Miglets (1973) produced experimental middle ear effusions in squirrel monkeys in such a way that the animals, having been sensitized passively with human serum containing a known reaginic antibody were challenged by insufflating the responsible allergen into the middle ears Miglets found in the middle ear mucosa of these squirrel monkeys, several morphological changes such as would be expected to occur in an antigen-antibody reaction, and suggested that the middle ear is an organ having susceptibility to allergic reactions However, based on the results of their own studies and of other investigators (Hotchkiss, 1948, Robison & Nickolas, 1951 Suehs, 1952), Senturia et al (1958) have stated that there is a lack of clear scientific evidence to support the claim that the fluid is an allergic fluid Our previous study (Mogi et al, 1974) resulted in the same conclusion Findings of the present study do not suggest that middle ear effusions result from the direct atopic al-

-ic reaction induced by mites present in the cous membrane lining the middle ear cavity l the Eustachian tube

t is doubtless so, that middle ear effusion is n in certain patients with allergy We agree h the opinion of Senturia et al (1958) that piratory tracts, including the middle ear vity and Eustachian tube, of patients with ergic diathesis have a greater susceptibility infection and that the allergic state in aller patients is a predisposing or conditioning tor rather than the cause of the effusion

ACKNOWLEDGEMENT

authors wish to thank Drs T Nakashima M Ogata Tawara and Y Onaka for providing specimens of ldle ear effusion The authors also gratefully acknowl e Shionogi Co Ltd Osaka Japan for providing RAST l RIST kits

ZUSAMMENFASSUNG

r haben Effusionssafte und Seren der beiden Mittel en von 56 Patienten die Otitis media mit den Kom ationen der Effusion ertrugen serologisch untersucht die Pathogenese von Effusion des Mittelohrs zu er ren Die Aktivitat des IgE Antikorpers gegen M lbe rde mittels des neulich entwickelten Radioallergo-bentstests (RAST) in vitro gemessen Die IgE Kon itration wurde auch durch den Radioimmunosorbents t (RIST) gemessen Die Resultate haben gezeigt daß IgE Antikorper gegen Milbe in funf Fällen Effusionen d in drei Fällen Blutseren bei den Patienten aufwies vier von fünf Patienten die RAST positiv waren rden Nasenallergie oder Symptome der Allergie be achtet Außerdem befanden sich in den zwei Fallen jenigen Effusionen die eine allergische Flussigkeit zu n schienen Von der oben erorterten Resultaten dieser itersuchungen ist es schwer zu sagen daß die Mittel reffusionen auf die unmittelbaren allergischen Reak nen in der Schleimhaut des Mittelohrs gegen Milben ruckzuführen sind

REFERENCES

S W 1962 Management of secretory otitis med a *Arch Otolaryngol 75* 130

R R A Howard A W & Mynors L S 1953 A serological procedure theoretically capable of de tecting incomplete and non precipitating antibodies to soluble protein antigens *Brit J Exp Path 34* 525

strom G Johansson G & Kiviloog J 1973 Im munoglobulin IgE concentrations in an adult clinical material with chron c obstructive pulmonary disease *Scand J Respir Dis 54* 73

Derlacki E L 1952 Aural manifestations of allergy *Ann Otol Rhinol Laryngol 61* 179

Donovan R Johansson S G O Bennich H H & Soothill J F 1970 Immunoglobulins in nasal polyp fluid *Int Arch Allergy Appl Immunol 37* 154

Fourcard T Berg T Johansson S G O & Wahren B 1971 Virus serology and serum IgE levels in children with asthmatoid bronchitis *Acta Paed atr Scand 60* 671

Hotchkiss W T 1948 Chronic secretory otitis *South Med J 41* 727

Ishizaka K & Newcomb R W 1970 Presence of IgE in nasal washings and sputum from asthmatic patients *J Allergy Clin Immunol 46* 197

Johansson S G O 1969 IgE in allergic diseases *Proc R Soc Med 62* 37

Johansson S G O Bennich H & Berg T 1971 *In vitro* diagnosis of atopic allergy III Quantitative estimation of circulating IgE antibod es by the radioallergosorbent test *Int Arch Allergy Appl Immunol 41* 443

— 1972 The clinical significance of IgE *Progr Clin Im munol 1* 157

Johansson S G O Bennich H & Wide L 1968 A new class of immunoglobulin in human serum *Immunology 14* 265

Jordan R 1949 Chronic secretory otitis media *Laryngo-scope 59* 1002

Lecks H I 1961 Allergic aspects of serous otitis media in children *N Y State J Med 61* 2737

Lim D J & Birck H 1971 Ultrastructural pathology of the middle ear mucosa in serous otitis media *Ann Otol Rhinol Laryngol 80* 838

McGovern J P Haywood T J & Fernandez A A 1967 Allergy and secretory otitis media *J A M A 200* 124

Miglets A 1973 The experimental production of allergic middle ear effusions *Laryngoscope 83* 1355

Miyamoto T Johansson S G O Ito K & Horiuchi Y 1974 Atopic allergy in Japanese subjects Studies primarily with radioallergosorbent test *J Allergy Clin Immunol 53* 9

Mogi G Honjo S Maeda S & Watanabe N 1974*a* Secretory immunoglobulin A (SIgA) in middle ear ef fusions A further report *Ann Otol Rhinol Laryngol 83* 92

Mogi G Honjo S Maeda S Yoshida T & Watanabe N 1974*b* Quantitative determination of secretory immunoglobulin A (SIgA) in middle ear effusions *Ann Otol Rhinol Laryngol 83* 239

— 1974*c* Immunoglobulin E (IgE) in middle ear effu sions *Ann Otol Rh nol Laryngol 83* 393

Mogi G Yoshida T Honjo S & Maeda S 1973 Middle ear effusions Quantitative analysis of im munoglobulins *Ann Otol Rhinol Laryngol 82* 196

Ogra P L Bernstein J M Yruchak A M Coppola P R & Tomasi T B 1974 Characteristics of secre tory immune system in human middle ear Implica tions in otitis media *J Immunol 112* 488

Okuda M 1974 Fundamental and clinical studies on nasal allergy *Otologica (Fukuoka) 20* Suppl 297

ages With human material, by necessity, only a scatter of ages can be obtained

MATERIAL AND METHODS

In this investigation 28 outbred albino laboratory guinea pigs (♂♂ and ♀♀) were used, 7 in each of 4 age groups <24 hours, 6 weeks, 3 months and 1 year In each age group the maximum number of animals from the same litter was two

Both cochleae of each animal were fixed in a veranol buffered solution of osmium tetroxide (1 3%) for 2 to 3 hours, washed in water and dehydrated in alcohol (70%) The whole spiral organ of each cochlea was examined by phase contrast microscopy after preparation by the surface specimen technique (Engstrom et al , 1966) The spiral organ was removed from the cochlea by microdissection under a binocular microscope and mounted in sections on slides Each section was labelled according to its position in the cochlea The regular geometric pattern of hair cells can readily be discerned under the phase contrast light microscope The three rows of outer hair cells (OHC) and the single row of inner hair cells (IHC) were mapped for the whole spiral organ f each ear using the cochleogram described Coleman (1975) Hair cells were recorded s damaged if the sensory hairs, the cell nucle us and the cell membrane had all degenerated The degeneration of a hair cell results in the formation of a distinct 'phalangeal scar Coch leographs were prepared by dividing each cochleogram into units of 100 OHC For each of these units the percentage number of OHC intact in each row was counted and plotted against the length of the spiral organ, measured as cumulative number of OHC

In the statistical analysis of the data each animal was taken as the basic experimental unit, i e the data from the right and left ears were averaged so that in each sample (age group) there were seven independent ob servations This avoided the problem of deal ing with correlated observations within each sample

Table I *Means and standard deviations of % number of OHC damaged*

Age		OHC 1	OHC 2	OHC 3	Total OHC
1 day	$\bar{x}$	0 27	0 50	0 66	0 45
	S D	0 15	0 18	0 33	0 12
42 days	$\bar{x}$	0 96	2 44	2 14	1 85
	S D	0 44	2 05	1 19	1 06
90 days	$\bar{x}$	1 49	3 23	4 91	3 19
	S D	0 63	1 37	2 10	1 27
365 days	$\bar{x}$	5 00	6 43	9 11	6 8?
	S D	3 03	3 49	3 96	3 13

RESULTS

The means and standard deviations of the percentage number of OHC damaged per animal are recorded in Table I It is apparent from this table that in each age group the loss of OHC is minimal in the first, or inner row of OHC (OHC 1) In all age groups the loss is maximal in the third, or outer row (OHC 3) with the exception of the 6-week group in which the maximum loss is in the second row (OHC 2) In all the ears studied, at all four ages, the loss of OHC was greatest towards the apex of the cochlea

Age <24 hours

In the animals aged <24 hours the number of damaged OHC per cochlea ranged from 17 to 51, the percentage number of OHC damaged per animal ranged from 0 34% to 0 68% with a mean of 0 45% The number of IHC dam aged per cochlea ranged from 0 to 8, with a

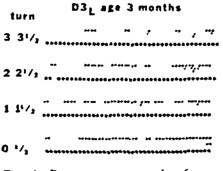
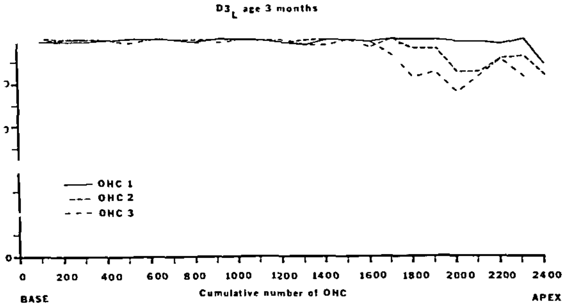


Fig 1 Representative samples from a cochleogram showing damaged hair cells in the cochlea of an animal aged 3 months



2 Cochleograph of the spiral organ of a 3 month-old showing % OHC intact plotted against the length he spiral organ

ı of 2 7 In all the ears which had some ;ree of IHC loss this loss was in the apical of the cochlear spiral

6 weeks

the animals aged 6 weeks the number of maged OHC per cochlea ranged from 52 270 This corresponded to a range of 0 93% 3 86% per animal, with a mean of 1 85% he average number of damaged IHC per lea was 2 2, with a range of 0 to 7 In ears this IHC loss was confined to the al turn while in 4 ears damaged IHC also found elsewhere in the cochlea

3 months

he number of damaged OHC in the 3-month 's ranged from 96 to 392 per cochlea he mean percentage number per animal in to 3 19% in this age group, with a of 1 91% to 5 57% The mean number damaged IHC remained close to that of the previous groups at 2 57, with a range of to 7 This IHC loss was fairly evenly distributed in most cochleae Fig 1 shows four representative samples from a cochleogram of the left ear of a 3 month old guinea pig It can be seen that OHC loss is greatest in the third row, with fewer lost from the OHC 2, and none from the OHC 1 The loss of OHC is also greatest at turns 2–2½ and 3–3½, with very few missing nearer the base A typical cochleograph of the ear from a 3 month old animal is illustrated in Fig 2 It is evident that the loss of OHC is maximal at a region near to the apex, and not at the apical tip itself This near-apical region of maximum OHC loss is typical of 3 month and 1 year old ears Fig 3 shows the average cochleograph prepared from the data from both ears of all the 3 month old animals The percentage number of OHC intact declines at the apical end of the spiral organ, and this reduction in number is most marked in the third row, then the second row, and is least in the first row

Age 1 year

In the 1 year old animals the number of damaged OHC ranged from 127 to 761 per ear

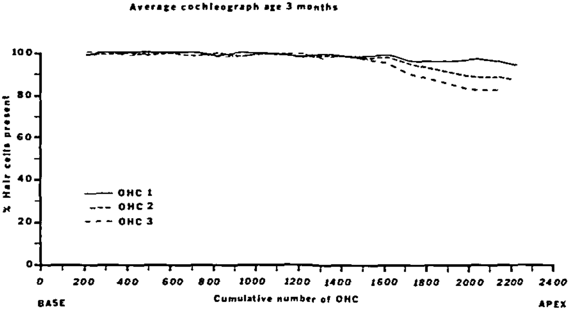


Fig. 3. Average cochleograph from the ears of all the 3 month-old animals.

The mean percentage number damaged per animal increased to 6.28% with a range of 2.85% to 11.1%. The mean number of damaged IHC increased from that of the other age groups to 4.1, with a range from 0 to 9. Fig. 4 shows a photomicrograph of a surface view of the spiral organ at a region between turns 2½ and 3. The row of IHC is complete. There is a single degenerated OHC in the inner row, 5 adjacent scarred OHC and 3 adjacent

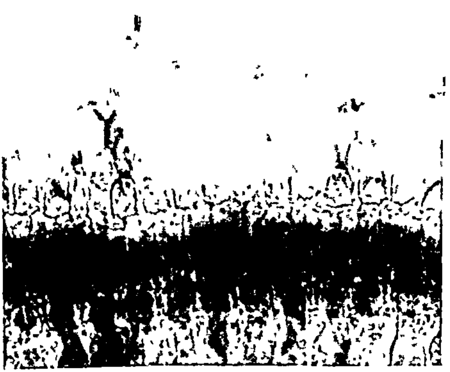
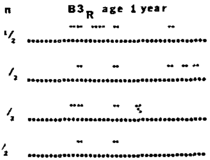


Fig. 4. Hair cell loss in an animal aged 1 year between turns 2½–3 of the spiral organ. Note large number of scars in the second row of OHC. ×1050.

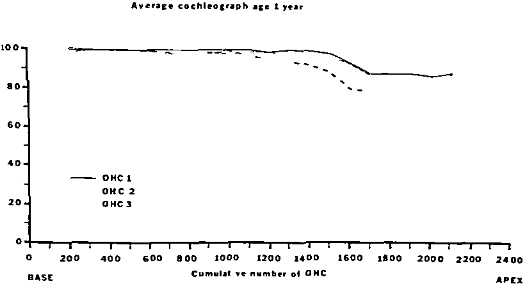


5 Representative samples from a cochleogram ving damaged hair cells in the ear of an animal aged ar

ed OHC in the second row and about 5 ittered scars in the third row This degree hair cell loss is common in this region of spiral organ in animals aged 1 year Even 6-week and 3 month old animals 6 or more jacent phalangeal scars are commonly found ether especially in the outermost row g 5 shows four samples taken from the chleogram of the right ear of a 1 year old ani l Again the loss of hair cells is greatest rther from the spiral modiolus and towards e apex of the cochlea Fig 6 shows the erage cochleograph taken from both ears of all the 1 year old guinea pigs This average cochleograph differs from that of the 3 month old animals in several ways the loss of OHC is more advanced and also starts nearer the base of the cochlea (especially the OHC 3) In this average cochleograph the near apical region of OHC loss is more apparent, whereas in Fig 3 the dip which was present in many ears, became obscured in the averaging of the data

Statistical analysis

The statistical significance of the observed trend of increase in degree of outer hair cell loss with increase in age was ascertained by Jonckheere s analysis of variance a non parametric (distribution–free) *k* sample test against ordered alternatives (Jonckheere 1954) This test gave a value of $S=252$ $p=<0\ 000001$ for sample sizes $m=7$ This result is very highly significant the four samples differ with the means occurring in a specific monotonic order with age The four samples were non homoscedastic i e they had non equal variances In fact the variance and hence the standard deviations increased with increase in age (Table I)



g 6 Average cochleograph from the ears of all the year-old an mals

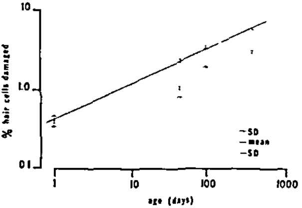


Fig 7 The percentage number of OHC damaged per animal with means and means±standard deviations plotted against age

The closest fit to a linear relationship between age (measured in days) and mean percentage number of OHC damaged was obtained by plotting both variables on logarithmic scales In Fig 7 the percentage number of OHC damaged per animal is plotted against age, using logarithmic scales for both abscissa and ordinate Included on this graph are the means and means±standard deviations of the % OHC damaged per age group, and a line estimated by linear regression analysis of the data The coefficient of product-moment correlation was calculated for log values of mean % damage and log values of age, which gave $r=0\ 997$, $p=0\ 002$ for 2 degrees of freedom Thus the degree of outer hair cell loss (mean % damaged) increases as a power function of age

A linear regression analysis was conducted on the log values of mean % OHC damaged per sample, and log values of age, to determine the straight line which best represents the observed data The resulting regression line of 'best fit' is included in Fig 7 The line is described by the equation

$$\log y = -0\ 382 + 0\ 454 \log x \qquad (1)$$

where y = % OHC damaged x = age, measured in days The % OHC damaged can therefore be described as a power function of age according to the equation

$$y = 0\ 415\, x^{0\ 454} \qquad (2)$$

Jonckheere's test was used again to ascertain whether the apparent increase in degree of outer hair cell loss from row 1 to row 2 to row 3 was a real one in the group of 1 year old animals The result was significant $S=59$ $p=0\ 03$, for sample sizes $m=7$

DISCUSSION

The results of this investigation show that degeneration and loss of outer hair cells commences at, or before, the time of parturition in the guinea pig This loss of OHC continues at a decelerating rate, as a power function of age (eq 2) In many guinea pigs aged only 6 weeks or 3 months there is often a considerable degree of OHC loss In contrast, degenerated inner hair cells are much less frequent, especially in animals aged 3 months or less Federspil (1972) used the surface specimen technique to study the normal pattern of hair cells in the spiral organs of 2 month old guinea pigs He found OHC loss was greatest at the apex where it reached an average of 1 08% Ulehlova (1973) used the same technique to study pigmented guinea pigs aged 6 weeks and 3 years In the 6-week old animals an average of 0 73% of OHC were found degenerated, and in the 3 year old animals 8 59% The guinea pigs used in the present study suffered a greater degree of hair cell loss than that described in the earlier reports an overall average of 1 85% of OHC damaged per cochlea was found at the age of 6 weeks Federspil and Ulehlova both described maximal hair cell loss at the apex of the cochlea In many of the animals studied here, the position of maximal loss was at a near-apical region (Figs 2, 6)

Covell & Rogers (1957) used serial sectioning to study the condition of the ears of guinea pigs between the ages of 2 and 4½ years The main feature of these ears was loss of spiral ganglion cells and peripheral nerve fibres at the apex of the cochlea These authors failed to detect changes in the spiral organ until

l degeneration had reached an advanced

'e microphonic (CM) and action po-
ial (AP) responses recorded from the
' window have been reported to be con
ly reduced in aged guinea pigs (Pesta-
et al , 1957) However, it is unlikely that
reduction was due to loss of hair cells,
he technique which these workers used
only sensitive to bio-electric signals
ı the lower half of the basal turn of the
ılea, where hair cell loss was probably
slight Crowley and associates (1972)
ured CM and AP responses to loud
from the round windows of rats aged 1,
12, 18 and 24 months CM sensitivity
d a peak at the age of 12 months, and
declined in older animals AP sensitivity
ained fairly constant up to 12 months,
then also declined in older animals Loss
inner and outer hair cells increased with
and was consistent with the changes in
and AP sensitivity However, as Crow-
points out loss of hair cells may have
ı one of many factors contributing to the
changes in auditory evoked re
onses

According to Heffner et al (1971) guinea
show a deterioration in hearing threshold
nsitivity to all sound frequencies with in
ease in age as determined by behavioural
metry

The near apical loss of outer hair cells and
minimal degree of inner hair cell loss in guinea
igs differs markedly from the pattern of
cell loss characteristic of human ears
ıg (1968) and Johnsson & Hawkins
1972) found that in aged patients OHC loss
as maximal at the base and at the apex of
cochlea in most ears while in some ears
HC loss was fairly diffuse IHC loss was
to occur mainly in the basal turn and
invariably associated with loss of pe
nerve fibres

In a survey of the literature between
and 1972 Falk and associates (1973)
luded that too often workers failed to
examine controls adequately when using the surface specimen technique The need to use adequate controls is further emphasised by the present observation that hair cell loss is often quite marked, even in young animals There is also a considerable amount of inter-strain, and perhaps also inter-stock variation in the normal pattern of sensory hair cells in guinea pigs of different ages (cf Federspil, 1972, Ulehlova, 1973)

ACKNOWLEDGEMENTS

The author would like to thank Dr A Pye for valuable discussion and also Mr R I Damper for advice concerning the statistical analyses reported in this paper

ZUSAMMENFASSUNG

Die Oberflachenpraparation und Cochleogramme wurden benutzt um die beiden Cortischen Organe bei 28 normalen Meerschweinchen in vier Altersgruppen zu uberprufen 7 im Alter von <24 Stunden 7 im Alter von 6 Wochen 7 im Alter von 3 Monaten und 7 im Alter von 1 Jahr Die Zahl der geschadigten Haarzellen wurde bei jedem ganzen Cortischen Organ aufgezeichnet Bei <24 Stunden alten Meerschweinchen betrug der Mittelwert der geschadigten außeren Haarzellen 0 45% bei 6 Wochen alten Meerschweinchen 1,85% bei 3 Monaten alten Meerschweinchen 3 19% und bei 1 Jahr alten Meerschweinchen 6 82% In allen Altersgruppen war die Hochstzahl der fehlenden außeren Haarzellen in der dritten Reihe und gegen die Spitze des Cochleas Der Schwund an inneren Haarzellen was sehr gering Die Hochstzahl der geschadigten inneren Haarzellen pro Cochlea war 9

REFERENCES

Bredberg G 1968 Cellular pattern and nerve supply of the human organ of Corti *Acta Otolaryngol* (Stockh) Suppl *236*

Coleman J W 1975 Hair cell ratios in the spiral organ of the guinea pig *Br J Audiol 9* 19

Covell W P & Rogers J B 1957 Pathological changes in the inner ears of senile guinea pigs *Laryngoscope 67* 118

Crowley D E Schramm V L Swain R E & Swanson S N 1972 Analysis of age related changes in electric responses from the inner ear of rats *Ann Otol Rhinol Laryngol 81* 739

Engstrom H Ades H W & Andersson A 1966 *The Structural Pattern of the Organ of Corti* Almqvist & Wiksell Stockholm

Falk S A Haseman J K Klein R & Falk M K 1973 Methyl mercury ototoxicity a model for the surface specimen technique *Laryngoscope 83* 1769

Federspil P 1972 Das Cochleogramm des normalen Meerschweinchens *Arch Kl n Exp Ohren Nasen Kehlkopfheilkd 201* 283

Heffner R Heffner H & Masterton B 1971 Behavioural measurements of absolute and frequency d fference thresholds in guinea pig *J Acoust Soc Am 49* 1888

Johnsson L G & Hawkins J E 1972 Sensory and neural degeneration with aging as seen in microdissection of the human inner ear *Ann Otol Rhinol Laryngol 81* 179

Jonckheere A R 1954 A distribution free K sample test against ordered alternatives *Biometrika 41* 133

Kohonen A & Tarkkanen J 1969 Cochlear damage from ototoxic antib otics by intratympanic application *Acta Otolaryngol* (Stockh) *68* 90

Pestalozza G Davis H Eldredge D H Covell W P & Rogers J B 1957 Decreased bio-electric potentials in the ears of senile guinea p gs *Laryngoscope 6* 1113

Stockwell C W Ades H W & Engström H 196 Patterns of hair cell damage after intense auditory stimulation *Ann Otol Rhinol Laryngol 78* 1144

Ulehlova L 1973 Normal cellular pattern of the organ of Corti in the guinea pig *Arch Klin Exp Ohren Na Kehlkopfheilkd 204* 321

J W Coleman B Sc
The Institute of Laryngology and Otology
330 Gray s Inn Road
London W C 1
England

Acta Otolaryngol 82 41–47, 1976

EFFERENT NERVE FIBERS ASSOCIATED WITH THE OUTERMOST SUPPORTING CELLS OF THE ORGAN OF CORTI IN THE GUINEA PIG

C G Wright and R E Preston

From the Kresge Hearing Research Institute Department of Otorhinolaryngology University of Michigan Medical School Ann Arbor Mich USA

(Received August 7 1975)

stract In whole mount preparations of the organ of orti nerve fibers are sometimes seen peripheral to the ird row outer hair cells where they appear to wander ong the Hensen cells These fibers were studied in e guinea pig by both light and electron microscopy In e upper second third and fourth cochlear turns nerve bers were seen between and on the peripheral side of e third row Deiters cells as well as in the floor of the uter tunnel space They were not found in the inter llular spaces between the Hensen cells These nerve bers are continuous with the spiral efferent fibers be eath the outer hair cells Also their ultrastructural and istochemical characteristics indicate that they are ef rent neural elements This identification was confirmed y the finding that they degenerate following interruption f the olivo-cochlear bundle in the brain stem

uring the course of a recent study concerning he cochlear efferent nerve supply in the inea pig, fine, beaded nerve fibers were seen n the neighborhood of the Hensen cells Wright & Preston, 1973) These fibers were resent in the upper turns of the organ of Corti ınd had been darkly stained by the zinc io- ide-osmic acid (ZIO) method

Occasional mention has been made of such ibers in a variety of animals since the early 1900 s In 1901 Kishi described nerve fasci cles lying peripheral to the outermost row of Deiters cells under the Hensen cells of the rabbit In well stained preparations from the mouse, Kolmer (1927) found isolated nerve bundles ' between" the Hensen cells

More recently, Engstrom et al (1966) ob served nerve fibers stained by the ZIO tech nique in the floor of the outer tunnel space of guinea pigs They speculated that such fi- bers might be autonomic in nature On the other hand, Iurato & Smith (1973) noted ace tylcholinesterase positive fibers among the Hensen cells of the guinea pig and concluded that they were part of the efferent nerve supply

The present study was undertaken to pro- vide more positive identification of these neural elements and to clarify their anatomical relationship to the cells on the periphery of the organ of Corti in the guinea pig

METHODS

The light and electron microscopic observa tions presented in this report were made on cochlear tissue obtained from young guinea pigs (200–300 gm) Fourteen animals received brain stem lesions designed to interrupt the efferent nerve supply to the inner ear Eleven of these were midline lesions which severed the crossed olivo-cochlear bundle and three were lateral lesions which successfully cut both the crossed and uncrossed portions of the efferent system

Support for this work was provided by the Deafness Research Foundation NINDS USPHS Research Grant 05065 and Program Project Grant 05785

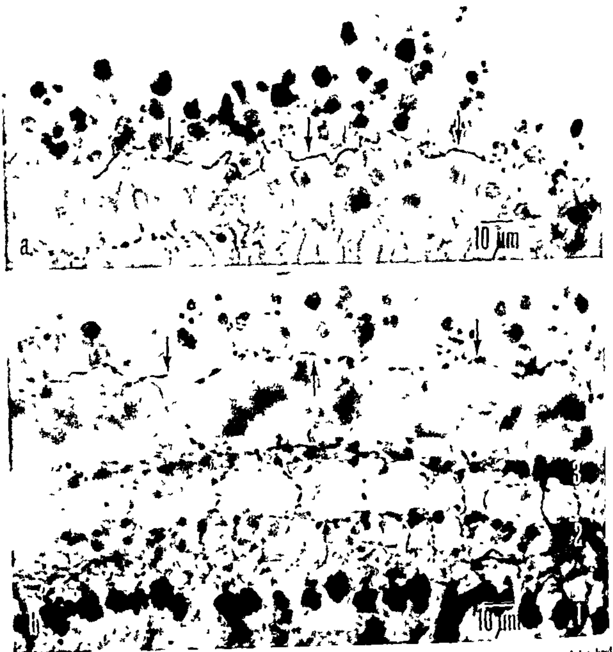


Fig 1 Light micrographs of ZIO-stained nerve fibers (arrows) coursing near the Hensen cells in the upper third turn of the guinea pig cochlea (*a*) Thick section (1 μ) showing a nerve fiber with a meandering course adjacent to the Hensen cells, which contain osmiophilic lipid droplets (*b*) Similar fiber in a whole mount preparation in which nerve endings beneath the three rows of outer hair cells (1 2 3) are visible

Except for one animal with a 48-hour survival time which was studied by electron microscopy, the experimental animals were permitted to survive for periods of 8 to 41 days in order to allow sufficient time for maximum degeneration of efferent neural elements in the organ of Corti The survival times allowed each of the animals are shown in Table I

Cochlear tissue from 13 of the above animals and from normal, unoperated guinea pigs was stained by the zinc iodide-osmic acid method for study by light microscopy. The procedures used for the brain stem lesions,

Table I

Postoperative	Number of animals	
Survival time (days)	Midline lesions	Lateral lesions
2	1	–
8	–	1
10	2	–
15	2	1
18	1	–
21	–	1
28	2	–
30 40 41	1 ea	–

histological controls, and nerve staining are described in a previous publication (Wright & Preston, 1973)

Electron microscopic observations were made on fragments of the organ of Corti from 3 normal guinea pigs and from one of the experimental animals which had a midline lesion with postoperative survival time of 48 hours The tissue was prepared for electron micro scopy according to the methods given in the article cited above

RESULTS

In whole mount preparations of the organ of Corti stained by the ZIO method, nerve fibers can sometimes be seen in a location peripheral to the third row of outer hair cells These fibers are usually found fairly close to the basilar membrane at about the level of the floor of the outer tunnel space As illustrated in Fig 1, they often appear to be wandering among the Hensen cells They are present only in the upper second, third, and apical cochlear turns of the guinea pig

When carefully traced these fibers can be found to be continuous with the spiral efferent nerve fibers beneath the outer hair cells They are formed by multiple branches and arcades extending outward from the fibers under the hair cells In most cases they are elaborations of the innervation associated with the third row hair cells However, some are extensions of fibers beneath the first or second row of hair cells

The density of the innervation in the neighborhood of the Hensen cells varies considerably from one animal to another In some cases only occasional nerve fibers are seen, in others, an almost plexus like arrangement of many fine fibers is present as shown in Fig 2

Study of radial sections of the organ of Corti by both light and electron microscopy has helped to clarify the relationship of the nerve



Fig 2 ZIO stained nerve fibers (*arrows*) extending into the neighborhood of the Hensen cells in the lower apical turn *SE* spiral efferent fibers beneath outer hair cells

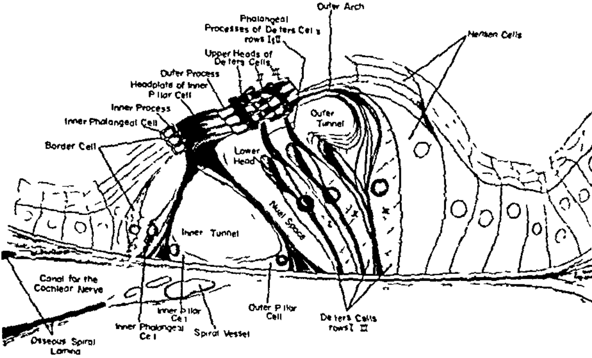


Fig 3 Diagram by Held (1926) showing supporting cells of the organ of Corti The shape and position of the third row Deiters cells are clearly depicted in this illustration The Hensen cells like the unlabelled inner sulcus cells are drawn in a highly schematic fashion The terms ou[illegible] *process* and *inner process* correspond to the Germa[illegible] *Aussenruder* (lit outrigger) and *Innenschnabel* (lit inne[illegible] beak)

ers to the outermost supporting cells of the cochlear receptor In the upper turns, the phalangeal processes of the third-row Deiters cells arch outward against the innermost Hensen cells to form the peripheral boundary of the outer tunnel space Also, the bulk of the cell body of each Deiters cell is situated peripheral to the hair cells of the third row The close anatomical relationship of the outermost D[illegible]

This relationship has been confirmed with the electron microscope by Engstrom & Wersall (1958) and by Iurato (1961)

We found that nerve fibers are present not only in the floor of the outer tunnel but also run between the third row Deiters cells to reach the intercellular spaces between the Deiters and Hensen cells We have not observed them peripheral to this point i e within the intercellular spaces between the Hensen cells themselves Rather, they are located between and on both the modiolar and peripheral sides of the Deiters cells of the third row The location of the nerve fibers with respect to the supporting cells is best demonstrated by electron microscopy and is illustrated in the micrographs shown in Fig 4

The nerve fibers near the periphery of the organ of Corti are positively stained by both the ZIO and acetylcholinesterase methods (Wright & Preston, 1973, Iurato & Smith 1973), which indicates that they belong to the efferent nerve supply. Their efferent nature was verified in the present study by the finding that they degenerate after the efferent innervation is surgically interrupted in the brain stem

In the three animals we studied which had successful lateral brainstem lesions that severed both components of the cochlear efferent nerve supply, no normal efferent neural elements were found in the organ of Corti In

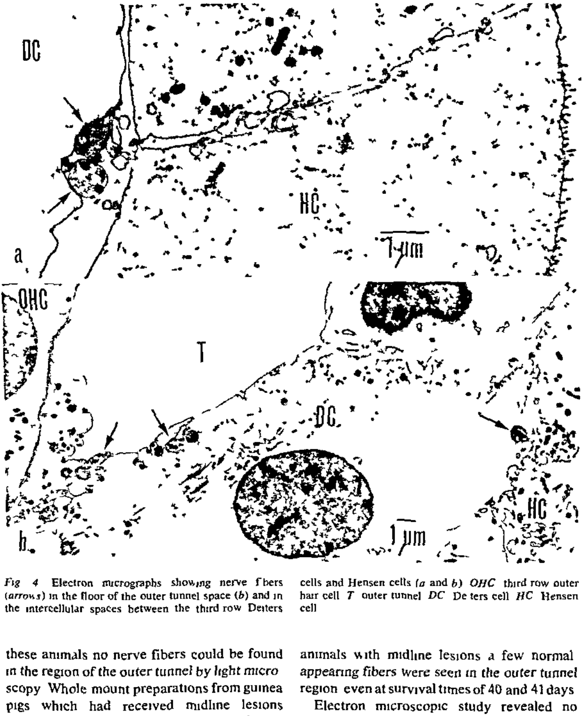


Fig 4 Electron micrographs showing nerve fibers (*arrows*) in the floor of the outer tunnel space (*b*) and in the intercellular spaces between the third row Deiters cells and Hensen cells (*a* and *b*) *OHC* third row outer hair cell *T* outer tunnel *DC* Deiters cell *HC* Hensen cell

these animals no nerve fibers could be found in the region of the outer tunnel by light microscopy Whole mount preparations from guinea pigs which had received midline lesions showed a definite reduction in the number of fibers present in this region The electron micrograph shown in Fig 5 illustrates a degenerating fiber located adjacent to a Hensen cell in an animal killed 48 hours after a midline brain stem lesion It should be noted that in animals with midline lesions a few normal appearing fibers were seen in the outer tunnel region even at survival times of 40 and 41 days

Electron microscopic study revealed no evidence of synaptic contacts formed by these outermost fibers with either the Deiters or Hensen cells The fibers do however display many vesiculated swellings along their length which are characteristic of efferent neural elements inside the organ of C

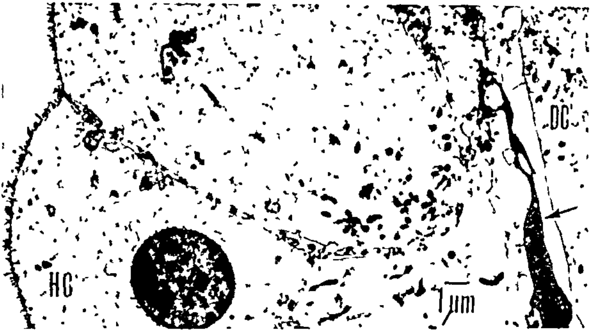


Fig 5 Degenerating efferent nerve fiber (*arrow*) adjacent to Hensen cells (*HC*) This fiber was found in an animal killed 48 hours after a midline brainstem lesion *DC* Deiters cell

DISCUSSION

When studied by light microscopy in whole mount preparations, the nerve fibers described in this report often appear to be coursing among the Hensen cells However, examination of radial sections by both light and electron microscopy shows that such fibers are actually more closely associated with the third row of Deiters cells, they have not been found to enter the intercellular spaces between the Hensen cells

These nerve fibers were found to degenerate following interruption of the cochlear efferent nerve supply in the brain stem If both the crossed and uncrossed portions of the efferent innervation were included in the lesion, there was a total disappearance of the fibers normally seen near the Hensen cells On the other hand, if only the crossed olivo cochlear bundle was cut there was a reduction in the number of these fibers but not a complete disappearance These findings indicate that both components of the efferent system supply nerve fibers which reach the area of the outer tunnel space in guinea pigs

In an earlier investigation of the distribution of the efferent nerve supply to the outer hair cells it was found that the efferent fibers are oriented predominately in a spiral fashion in the upper half of the guinea pig s cochlea (Wright & Preston, 1973) These spiral fibers tend to wander freely back and forth between the three rows of Deiters cells as they approach their terminations on the hair cells Since the Deiters cells of the third row are situated quite far peripherally, in close relation to the Hensen cells, the efferent neural elements associated with these cells would tend to be displaced outward to a position peripheral to the third row of outer hair cells The efferent nerve supply of the lower cochlear turns is distributed to the outer hair cells in a radial manner so that the nerve fibers approach the hair cells more directly and without a spiral course among the Deiters cells These differences in the pattern of distribution of efferent nerve fibers to the outer hair cells of the upper and lower portions of the organ of Corti may account for the fact that nerve fibers are not seen in the neigh

borhood of the Hensen cells in the basal cochlear turns

The idea that the presence of nerve fibers between the supporting cells on the periphery of the organ of Corti may be explained on purely morphological grounds is in harmony with the fact that no evidence of functional contacts made by these fibers could be found

It seems quite unlikely that any autonomic *sympathetic fibers are present near the periph*eral edge of the organ of Corti as suggested in 1966 by Engstrom et al since subsequent fluorescence studies have failed to reveal any adrenergic nerves inside the organ of Corti (Terayama et al , 1966, Ross 1971, Densert, 1974)

The finding that the ZIO stained fibers seen by light microscopy are efferent does not, of course preclude the possibility that some afferent fibers may also be present in the vicinity of the outer tunnel as postulated by Smith & Haglan (1973) Due to the selectivity of the ZIO stain, unmyelinated afferent fibers are not visible in the light microscope and with the electron microscope it is often difficult to distinguish afferent from efferent fibers Therefore the question of whether afferent fibers have extensions peripheral to the third row outer hair cells must remain open

ACKNOWLEDGEMENTS

The authors wish to thank Prof Joseph E Hawkins jr for his critical reading of the manuscript and for many valuable discussions relating to this study

We are also grateful to Ana Luisa Rodriguez James W Bruce Vernon T Maulbetsch and Robert L Combs for skillful technical assistance

ZUSAMMENFASSUNG

In Flachenpraparaten des Cortischen Organs konnen manchmal an der peripheren Seite der äußeren Haarzellen der dritten Reihe Nervenfasern beobachtet werden die zwischen den Hensenschen Zellen zu verlaufen scheinen Diese Fasern wurden im Meerschweinchen licht und elektronenmikroskopisch untersucht In der oberen zweiten der dritten und der vierten Windung wurden Fasern beobachtet die zwischen den Deiterschen Zellen der dritten Reihe und entlang deren Aussenseite verliefen sowie am Boden des außeren Tunnelraumes Sie wurden nicht in den interzellularen Raumen zwischen den Hensenschen Zellen gefunden Diese Nervenfasern gehen uber in efferente Spiralfasern unter den außeren Haarzellen Ihre ultrastrukturellen und histochemischen Eigenschaften weisen ebenfalls darauf hin daß es sich um efferente Nervenelemente handelt Diese Identifizierung wurde durch die Beobachtung bestatigt daß sie nach Unterbrechung des olivocochlearen Bundels im Hirnstamm degenerieren

REFERENCES

Densert O 1974 Adrenergic innervation in the rabbit cochlea *Acta Otolaryngol* (Stockh) *78* 345

Engstrom H Ades H W & Andersson A 1966 *Structural pattern of the organ of Corti* Almqvist & Wiksell Stockholm

Engstrom H & Wersall J 1958 The ultrastructural organization of the organ of Corti and of the vestibular sensory epithelia *Exp Cell Res* Suppl *5* 460

Held H 1926 Die Cochlea der Säuger und der Vogel ihre Entwicklung und ihr Bau In *Handbuch der normalen und pathologischen Physiologie* Band XI (ed A Bethe G von Bergmann G Embden und A Ellinger) pp 467–534 Springer Berlin

Iurato S 1961 Submicroscopic structure of the membranous labyrinth 2 The epithelium of Corti s organ *Zellforsch M krosk Anat 53* 259

Iurato S & Smith C A Unpublished observations cited in Smith C A and Haglan B J 1973 Golgi stains on the guinea pig organ of Corti *Acta Otolaryngol* (Stockh) *75* 203

Kishi J 1901 Uber den Verlauf und d e periphere Endigung des Nervus cochleae *Arch Mik Anat 59* 144

Kolmer W 1927 Gehororgan In *Handbuch der mikroskopischen Anatomie des Menschen* III *Haut und Sinnesorgane* (ed W von Mollendorff) pp 250–478 Springer Berlin

Ross M D 1971 Fluorescence and electron microscopic observations of the general visceral efferent innervation of the inner ear *Acta Otolaryngol* (Stockh) Suppl *286*

Smith C & Haglan B J 1973 Golgi stains on the guinea pig organ of Corti *Acta Otolaryngol* (Stockh) *75* 203

Terayama Y Holz E & Beck C 1966 Adrenergic innervation of the cochlea *Ann Otol Rhinol Laryngol 75* 69

Wright C G & Preston R E 1973 Degeneration and distribution of efferent nerve fibers in the guinea pig organ of Corti A light and scanning electron microscopic study *Brain Res 58* 37

C G Wright Ph D
Kresge Hearing Research Institute
Dept of Otorhinolaryngology
University of Michigan Medical School
Ann Arbor Mich 48109 USA

Acta Otolaryngol 82 48–56, 1976

HISTO PHYSIOLOGICAL RELATIONSHIPS IN THE DEAF WHITE CAT AUDITORY SYSTEM

G Rebillard, M Rebillard, E Carlier and R Pujol

From the Laboratory of Sensory Neurophysiology University of Provence Marseille France

(Received August 4 1975)

Abstract An investigation is underway in deaf white cats in order to clear up some problems about the timing of cochlear degeneration the relationships between physiological and histological findings and the consequential state of central auditory pathways The first results reported here show that degeneration of sensory structures was much more advanced than physiological results lead us to believe The possibility of direct fibre stimulation is discussed Although degeneration of nervous structures was delayed compared with sensory ones some early abnormalities in myelination were found In the adult completely deaf for years the electrical stimulation of cochlear nuclei evoked normal looking field responses at the cortical level Thus some degree of auditory pathway functional integrity appeared even long after they were disconnected from receptors

Hereditary deafness has interested scientists for years Alexander (1900) first reported a degenerative process of the cochlea as a main feature of this syndrome The cochlea seems to mature quite normally and often animals can hear, then it degenerates and they become deaf Among numerous papers, we can cite some on a variety of mutant mouses (Gruneberg et al , 1940, Deol 1954 1956, Mikaelian et al , 1965, 1974, Kikuchi & Hilding, 1965, 1967) Similar hereditary defects of hearing are frequently associated with pigmentation disorders such as a white coat or blue eyes That kind of abnormality has been described for the mink (Hilding et al 1967 Sugiura & Hilding, 1970), the dog (Anderson et al , 1968 Suga & Hattler, 1970) Even in man the Waardenburg s syndrome can be related to that matter (Waardenburg, 1951) But the most widely recognized species affected by this genetic impairment is the white cat Details on documentation can be found in the main papers of Bosher & Hallpike (1965), Suga & Hattler (1970), West & Harrison (1973) and Mair (1973*a*, *b* *c*, *d*)

In spite of this relatively large amount of data, there is still a lack of information about the critical stages of cochlear involution and the precise histo physiological relationships especially in the degenerating kitten not yet completely deaf Another problem arises in the adult white cat, with the neural auditory pathways residual state, long after they have been disconnected from the receptors The present paper gives the first results of an on going study in an attempt to elucidate these problems

MATERIAL AND METHODS

A pure strain of deaf white cat has been recently selected[1] and some of these animals have been taken for present investigations (3 adults and 3 few month old kittens)

After hearing tests were used in order to determine complete or partial deafness, cats were anesthetized by intraperitoneal injection

Supported by grants from INSERM (ATP 6-74-27) CNRS (ATP 1422) and DGRST (AC BRD)

[1] Through the Centre Régional d Elevage et de Production d Animaux de Laboratoire du CNRS (13790 – Rousset)

of nembutal Electrophysiological responses were tentatively recorded in the inferior colliculus and at the cortical level, after a large exposure of auditory areas Acoustic stimulation consisted of clicks or pure tones emitted monolateraly by a B & K 1 inch microphone or a Lansing loudspeaker connected to the ear bars of the stereotactic apparatus Secondly, when no physiological responses were found, electrical stimulation of the cochlear nuclei was used and evoked potentials were recorded at the cortical level

Histological techniques began by *in vivo* fixation of the cochlea, using osmium tetroxide After dissection of the temporal bones, cochleas were plunged into fixator fluid for 90 minutes Embedding in Epon or Spurr resin was done using the vacuum technique employed by Spoendlin (1975) With a Reichert OMU II microtome, transverse sections were made at various levels of the cochlea Semi thin sections were examined with a Reichert Zetopan microscope, after Toluidine blue staining or without staining for the Nomarski contrast Thin sections mounted on Formvar coated girds and counterstained with uranyl acetate and lead citrate, were checked with a Philips 300 electronmicroscope

RESULTS

Cats with incomplete deafness

Two out of the 3 kittens we investigated showed remnent hearing responses The most interesting one was a 4 month-old completely deaf on the right side, but still hearing on the left As a matter of fact it would be better to talk about poor hearing as responses could be elicited only by intense sounds (above 75 dB) and for frequencies ranging from 300 to 3 000 Hz

Light microscopy observations of that animal showed a completely degenerated cochlea on the right side (Fig 1) with absent sensory or neural elements and disorganized supporting cells The only clearly recognizable structures were tectorial membrane and Reissner's membrane plastered against the stria vascularis, the promontory and the remaining Corti's organ On the other hand, left cochlea, i e the still hearing one, was in a better stage of preservation especially at the apex (Fig 2) There, the cochlear ductus was still open, while at the base (Fig 3) it had completely collapsed as on the other side (cf Fig 1) Nevertheless, along the whole cochlea the sensory receptors were rather severely degenerated The best image of what could be an inner hair cell and a tunnel of Corti is seen in Fig 4 No recognizable structure whatsoever was found at the outer hair cell level (Fig 5) With regard to neural elements inside the cochlea the first approach showed a well preserved spiral ganglion with afferent and efferent myelinated fibres (Fig 6) and quite normal lamina spiralis fibres (Fig 4)

Electron microscopy findings first confirmed the light microscopy results Precisions were given as to stria vascularis degeneration An example is seen in Fig 7 where the stria of the left hearing cochlea appeared rather abnormal with two very thin layers of cells and a poor vascularisation In comparison, the stria on the deaf side (Fig 8) had only one layer of cells and it was covered by Reissner's membrane closely apposed to its surface This double-layer membrane was found to be similarly adhering to the remaining sensory or supporting cells (Figs 9, 10)

The most interesting results are those concerning neural elements at the level of the left cochlea Neurons in the spiral ganglion appeared normal at any level of that hearing cochlea (Fig 11) with the characteristic myelin sheaths (Fig 12) But when checking nervous fibres inside the lamina spiralis of which most are preganglionary a surprising number of non myelinated fibres was noted (Fig 13) As no degenerated material appeared, those fibres seemed to have never been myelinated at all

Another striking feature was found in the

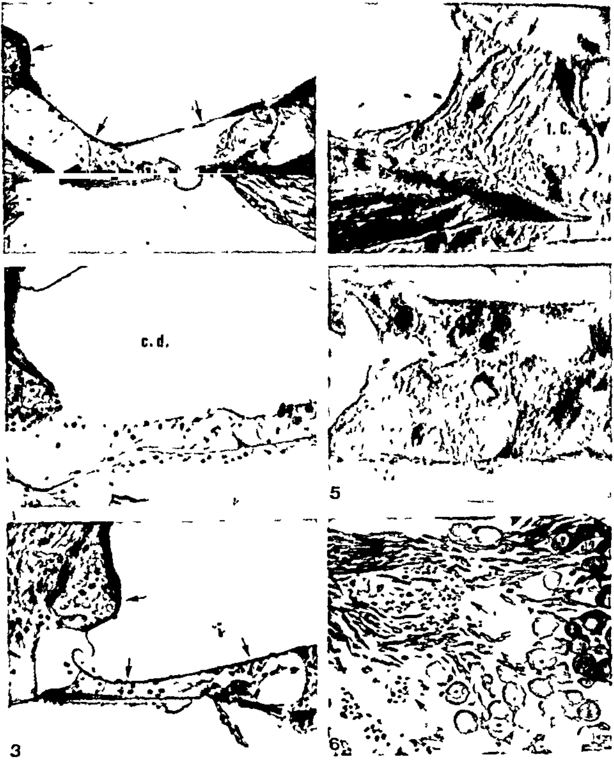
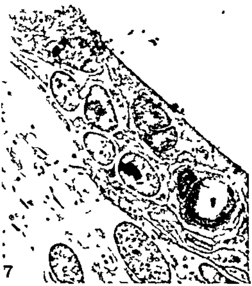


Fig 1 Deaf cochlea (*right*) from a 4 month-old kitten Cochlear degeneration is total with collapse of Reissner's membrane (*arrows*) on vestigial sensory and supporting structures Tectorial membrane and spiral vessel are visible Semi thin Nomarski ×150

Fig 2 Upper portion of the left hearing cochlea from a 4 month-old kitten Notice the cochlear ductus still open (*c d*) and the great disorganization of sensory structures Semi thin Toluidine blue staining ×150


7


8


9


10

Fig 3 Lower portion of the same cochlea As in Fig 1 Reissner s membrane is applied on stria and Corti s organ cochlear ductus is no longer seen Semi thin Toluidine blue staining ×150

Fig 4 One of the best preserved hair cells (inner one marked by arrow) found on the left cochlea Tunnel of Corti (*t C*) is still recognizable Notice the normal looking fibres inside the lamina spiralis Semi thin Nomarski ×600

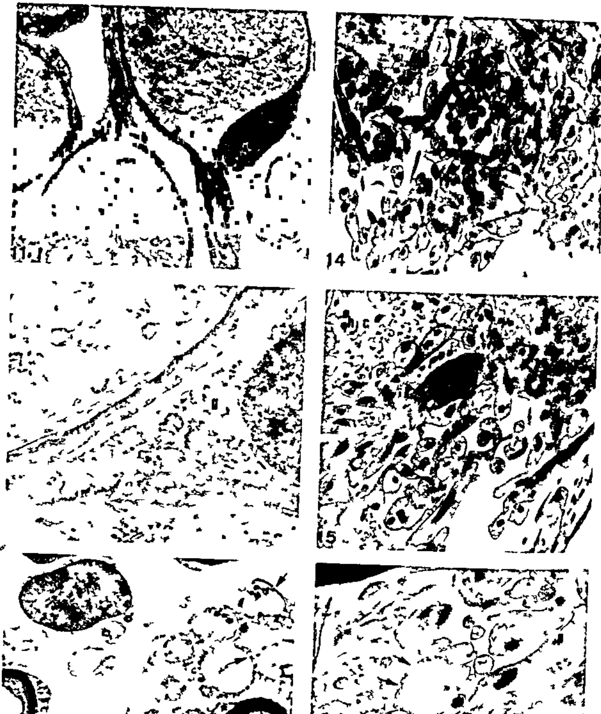
Fig 5 Level of outer hair cells of the same portion of the cochlea Degeneration is much more advanced Semi thin Nomarski ×600

Fig 6 Spiral ganglion of the left hearing cochlea from a 4 month old kitten Notice the myelination of neurons and of afferent and efferent (*arrows*) fibres Semi thin Nomarski ×240

Fig 7 Stria vascularis from the left hearing cochlea of a 4 month old cat Atrophy is noticeable especially at the inner layer level Blood vessels (v) appeared less numerous than normally ×3 200

Fig 8 Stria vascularis from an adult deaf white cat Atrophy is total with only one cell layer Reissner s membrane (outlined by arrows) has collapsed against stria Cochlear ductus no longer exists ×6900

Figs 9 and 10 Vestigial structures of Corti's organ from an adult deaf white cat at the level of outer hair cells (9) and Boettcher s cells (*10*) Notice Reissner's membrane (between arrows) lying against those structures ×6900



Figs 11 to 16 Electron micrographs f om the left hearing cochlea of a 4 month-old k en Glutaraldehyde-osmic ac d f xation

Fig 11 Normal look ng sp ral gangl on neurons and satel l te gl al cell (*g*) ×3 200

Fig 12 H gh magn f cat on of myel n sheath a ound sp ral gangl on neurons *g* nd cates a gl al cell ×18 000

Fig 13 Bundle of f bres ns de the lam na sp ral s Some are well myel nated others not at all except p omyel n lamellae (*a ows*) ×10 000

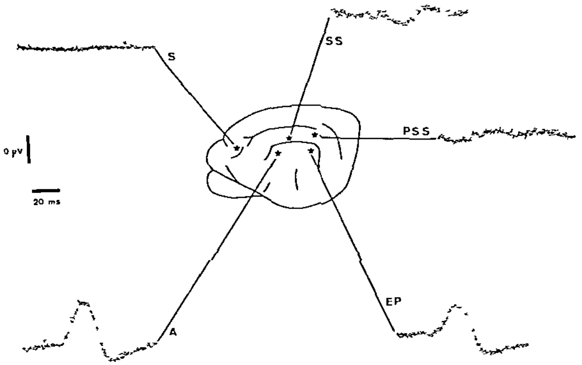


Fig 17 Adult deaf white cat Field-evoked potentials in response to electrical stimulation of cochlear nuclei Note the normal looking forms and latencies at primary (*A*) and extra primary areas (*EP SS*) No responses were recorded in somesthetic (*S*) or posterior suprasylvian (*PSS*) areas Positivity upwards

bundle of fibres reaching the degenerated Corti's organ after they passed through the habenula perforata There, although no recognizable sensory hair cells were distinguished, the number of fibres present was much greater than in normal preparations (Figs 14, 15) Some of these fibres appeared branched and others were filled with microvesicles (Fig 16)

Adult white cats with total deafness

When a bilateral complete deafness was found, i e , the impossibility of recording any potential after sound stimulation, animals were tested by applying an electrical stimulation to the first relay nuclei of the auditory pathway, i e cochlear nuclei This stimulation evoked field responses at a cortical level in the deaf cats that looked very similar to those in the control, normal-hearing ones Those responses (Fig 17) appeared only in the primary or extra primary areas, excluding other regions like somesthetic or visual ones Their forms and latencies varying with the location were also quite normal for example, a mainly positive response in the AI cortex with a 8 ms latency, and a negative response in the supra sylvian gyrus, with a 15 ms latency

The histology of such animals has not been completely studied Only cochlear preparations were investigated, showing sensory and neural degeneration like the one previously described on the non hearing side of a kitten (cf Figs 1, 8, 9, 10) No preparation of the brain structures has yet been checked

Figs 14 and 15 Nerve endings below vestigial hair cells (*h c*) Notice the great number of fibres some of them appearing branched ×6800 (14) ×10000 (15)

Fig 16 High magnification of nerve endings A vesiculated one is marked by arrows *p* indicates a vestigial pillar of Corti ×20000

DISCUSSION

Critical stages of cochlear degeneration

The sequence of degeneration of otic structures has tentatively been described by many authors However descriptions vary so much that it seems difficult to talk about a general process of involution For example in the mutant mice that process is said to begin at the level of the organ of Corti (Deol 1956) the stria vascularis (Gruneberg et al 1940 the spiral ganglion (Deol 1954) the spiral vessel (Kikuchi & Hilding 1967) and even at the level of the tectorial membrane (Deol 1954) In the white cat (Wilson & Kane 1959) as in the *Hedlund* white mink (Hilding et al 1967) the primary factor of atrophy is assumed to be a failure in endolymph formation in that respect degeneration of the stria vascularis and sagging of Reissner s membrane are considered as the earliest occurring abnormalities (Bosher & Hallpike 1965)

From the observations reported here it is impossible to give a definitive sequence but several facts can be pointed out First the degeneration of sensory cells may not be the earliest phenomenon but is seems to happen very early at a stage when the cochlear ductus is still wide open (see Fig 2) As is generally assumed (Mair 1973*c*) the atrophy affects outer hair cells before the inner ones Then the tectorial membrane and spiral vessel degeneration does not appear as a drastic phase since those structures can be found several months or years after completion of the involutive process The last point is about neural structures There is no doubt about a delayed degeneration of fibres and of spiral ganglion neurons (Mair 1973*d*) but one must keep in mind the unmyelinated fibres found in the early stages (see Fig 9) If we cannot consider that as degeneration we must talk about a primitive atrophy of nervous elements To conclude on this very controversial point one can turn to genetics The syndrome causing white fur and deafness results from a dominant autosomal gene appearing on structures developing from the neural crest (Bergsma & Brown 1971 Brown & Chung 1971) Then it is not too surprising to find the abnormality occurring at the same time at the level of pigmented cells of stria as well as in glial cells

The timing of cochlear involution in the white cat is another problem not yet completely cleared up Bosher & Hallpike (1965) who studied a total of 58 abnormal kittens gave the best approximation They think the involutive process begins as early as 5 postnatal days and is virtually complete by the 21st day From the other available data things do not appear so clearly Mair (1973*c*) agrees with an early beginning at the end of the first week but he states that degeneration is progressive throughout the animal s life span With the very few animals we have investigated up to now it seems difficult to opt for one or the other theory but we tend towards a very variable process which can in some cases be as short as 2 or 3 weeks and may last several months in others Another hypothesis could be a degeneration more or less complete depending on the animals even on the ear of the same cat In that case the variety of involutive stages described would be only the different kinds of terminal features

Anatomo functional relationships

Whatever the timing of the involutive process may be there is no doubt about the functional possibilities of abnormal white kittens before they reach the complete deaf stage Furthermore if the date of first abnormalities is taken into account that is at 5 or 6 days (Bosher & Hallpike 1965 Mair 1973*c*) it is possible to say that kittens have normal hearing for a week since onset of auditory function in this animal has been described around birth (Pujol & Marty 1970 Pujol & Hilding 1973)

After the degeneration has begun precise relationships have to be made between what remains from hearing abilities and the cochlear structures Some data underlined the con

rdance between the early degeneration of basal portion of the cochlea and the lack of sensitivity concerning first high frequency tones (Suga & Hattler, 1970) Apparently our results concur with this thesis as gross morphology and physiology were correlated But, we must keep in mind that, wherever it was along a cochlear partition, a normal-looking sensory structure was not found to be related to potentials

This last point opens a new discussion about which structures are responsible for residual hearing abilities Are there still some functional inner hair cells like the one shown in Fig 4? To confirm this hypothesis one needs a more sophisticated preparation of the cochlea, like the one described by Spoendlin (1972) and Spoendlin & Brun (1973) In the meantime, with regard to some of our results on nervous endings inside the degenerated cochlea, the idea of Suga & Hattler (1970) about some direct stimulation of neural elements by mechanical displacement cannot be rejected Our findings clearly indicated the presence of fibres, even more numerous than usual, at a quite degenerated cochlear level Some efferent vesiculated endings found at the same place could mark a working stage of such fibres deprived of normal junctions with hair cells

Consequential state of deafferented auditory pathways

These very first results reported on the auditory pathway electrical stimulation in deaf white cats allow us to consider some integrity of neural circuitry, long after it was disconnected from receptors On that point, one of the few available data was presented by West & Harrison (1973) They indicated, in the adult deaf white cat, a light transneuronal degeneration in the form of reduced cell size, in the ventral cochlear nucleus and the superior olivary complex As far as we know nothing has been done at the cortical level Of course, normal field responses do not mean complete integrity of structures and one needs more histological as well as microphysiological informations However, the question is still open about a functional significance of that circuitry early out of service Is it non-functional at all or partly recuperated by some other sensation in the case of bilateral deafness, or by controlateral auditory information when deafness is monolateral?

These are some of the problems we are now trying to deal with, both in deaf white cats and in normal ones deafferented soon after birth

ACKNOWLEDGEMENTS

We wish to express thanks for technical assistance of M L Agate and Mrs M R Ibrahim Part of electro-acoustic work was done with help from Laboratoire de Mecanique et d'Acoustique (CNRS Marseille) Electron microscopy was performed using equipment of Unité de Recherches Neurobiologiques (INSERM Marseille)

RÉSUMÉ

Une etude est entreprise sur les chats blancs et sourds dans le but d apporter des précisions sur certains problèmes comme les differentes phases de la degenerescence cochleaire les correlations anatomo-fonctionnelles de cette degénerescence et le devenir des voies et des centres auditifs Les premiers resultats montrent que dans certains cas la degenerescence des structures sensorielles est bien plus avancee que les résultats physiologiques ne le laissaient prevoir La possibilite d une stimulation directe des fibres nerveuses est discutee Bien que la degenerescence des elements nerveux soit posterieure à celle des structures sensorielles on trouve dès le début une myelinisation anormale des fibres intra cochleaires Chez l adulte complètement sourd depuis des années la stimulation electrique des noyaux cochleaires evoque des reponses apparemment normales au niveau cortical Ainsi se manifeste une certaine integrite fonctionnelle des voies auditives longtemps après la mise hors service des recepteurs

ZUSAMMENFASSUNG

Zahlreiche Arbeiten uber die congenitale Taubheit der weißen Katze wurden bereits veroffentlicht es bleiben jedoch noch mehrere ungeloste Probleme Die vorliegende Arbeit enthalt einige Beobachtungen uber die Involution der Cochlea bei der jungen Katze Selbst in Fallen in welchen das Cortische Organ schon weitgehend degeneriert war konnten wir oft betrachtliche Funktionsreste feststellen Die Moglichkeit einer direkten Reizung der noch intakten cochlearen Nervenzellen wird diskutiert Eine weitgehende Erhaltung der Hörbahn wurde bei

schon seit mehreren Jahren völlig tauben erwachsenen Tieren mittels elektrischer Reizung der cochlearen Kerne aufgezeigt

REFERENCES

Alexander, G 1900 Zur vergleichenden pathologischen Anatomie des Gehororganes 1 Gehörorgan und Gehirn einer unvollkommen albinotischen weissen Katze *Arch Ohrenheilkd 50*, 159

Anderson, H, Henricson, B, Lundquist, P G, Wedenberg E & Wersäll, J 1968 Genetic hearing impairment in the dalmatian dog *Acta Otolaryngol* (Stockh), Suppl *232*, 1

Bergsma, D R & Brown, K S 1971 White fur, blue eyes, and deafness in the domestic cat *J Hered 62*, 171

Bosher, S K & Hallpike, C S 1965 Observations on the histological features, development and pathogenesis of the inner ear degeneration of the deaf white cat *Proc R Soc Lond* (Biol) *162*, 147

Brown, K S & Chung, K C 1971 Observations on the epidemiology, genetics and pathogenesis of deafness in children and lower mammals *Audiology 10* 234

Deol, M S 1954 The anomalies of the labyrinth of the mutants varitint waddler, shaker-2 and jerker in the mouse *J Genet 52*, 562

— 1956 The anatomy and development of the mutants pirouette, shaker-1 and waltzer in the mouse *Proc Roy Soc Lond* (Biol) *145*, 206

Gruneberg H, Hallpike, C S & Ledoux, A 1940 Observations on the structure, development and electrical reactions of the internal ear of the shaker 1 mouse *(Mus musculus) Proc Roy Soc Lond* (Biol) *129* 154

ilding D A Sugiura A & Nakai Y 1967 Deaf white mink electron microscopic study of the inner ear *Ann Otol Rhinol Laryngol 76* 647

Kikuchi K & Hilding D A 1965 The defective organ of Corti in shaker 1 mice *Acta Otolaryngol* (Stockh) *60* 287

— 1967 The spiral vessel and stria vascularis in shaker 1 mice *Acta Otolaryngol* (Stockh) *63* 395

Mair, I W S 1973*a* Hereditary deafness in the white cat I Hereditary and clinical aspect *Acta Otolaryngol* (Stockh) Suppl *314* 5

— 1973*b* Op cit II Vestibular function and histopathology *Acta Otolaryngol* (Stockh), Suppl *314* 36

Mikaelian D, Alford, B R & Ruben, R J 1965 Cochlear potentials and VIII nerve action potentials in normal and genetically deaf mice *Ann Otol Rhinol Laryngol 74* 146

Mikaelian D O Warfield, D & Norris, O 1974 Genetic progressive hearing loss in the C57/b16 mouse *Acta Otolaryngol* (Stockh) *77* 327

Pujol, R & Hilding, D 1973 Anatomy and physiology of the onset of auditory function *Acta Otolaryngol* (Stockh) *76* 1

Pujol, R & Marty, R 1970 Postnatal maturation in the cochlea of cat *J Comp Neurol 139* 115

Spoendlin, H 1972 Innervation densities of the cochlea *Acta Otolaryngol* (Stockh) *73*, 235

— 1975 Personal communication

Spoendlin H & Brun J P 1973 Relation of structural damage to exposure time and intensity in acoustic trauma *Acta Otolaryngol* (Stockh) *75* 220

Suga, F & Hattler, K W 1970 Physiological and histopathological correlates of hereditary deafness in animals *Laryngoscope 80* 80

Sugiura, A & Hilding D A 1970 Cochleo-saccular degeneration in Hedlund white mink *Acta Otolaryngol* (Stockh) *69* 126

Waardenburg P J 1951 A new syndrome combining developmental abnormalities of the eyelids eye brows and nose root with pigmentary defects of the iris and head hair and with congenital deafness *Am J Hum Genet 3* 195

West, C D & Harrison, J M 1973 Transneuronal cell atrophy in the congenitally deaf white cat *J Comp Neurol 151*, 377

Wilson, T G & Kane, F 1959 Congenital deafness in white cats *Acta Otolaryngol* (Stockh) *50* 269

G Rebillard M.D
Lab de Neurophysiologie Sensorielle
Université de Provence
Centre St-Jerome
F 13397 Marseille Cedex 4
France

Acta Otolaryngol 82 57–69, 1976

INTERCELLULAR JUNCTIONS IN THE ORGAN OF CORTI AS REVEALED BY FREEZE FRACTURING

S Iurato,[1] K Franke,[2] L Luciano,[3] G Wermbter,[3] E Pannese[4] and E Reale[3]

From the [1]Department of Bioacoustics of the University of Bari Italy the [2]ENT Clinic and the [3]Laboratory of Electron Microscopy Department of Anatomy School of Medicine Hannover FRG and the [4]Second Institute of Human Anatomy University of Milan Italy

(Received September 29 1975)

Abstract The junctions between the cells of the organ of Corti have been investigated with the freeze fracture method in the chinchilla inner ear Numerous gap junctions have been found among all the supporting cells particularly at the level of their basal part This means that the supporting cells have their cytoplasm electrotonically and metabolically coupled Gap junctions were not observed between supporting and sensory cells nor between sensory cells and nerve endings At this level the transmission should be chemically mediated

Extensive zonulae occludentes sealing apically the intercellular spaces of the organ of Corti have recently been demonstrated at the level of the reticular membrane in replicas of freeze fractured material (Iurato et al , 1976) The aim of this paper is to study the junctional morphology of the organ of Corti at a deeper level by using the same method To confirm the results obtained with the freeze fracture technique the structure of the junctions in the organ of Corti was also studied by using colloidal lanthanum as an extracellular stain (Revel & Karnovsky, 1967)

MATERIAL AND METHOD

Twelve adult chinchillas of both sexes were used for this study

Research financed (partly) by the Consiglio Nazionale delle Ricerche grants (Nos 74 00210 04 115 2277 and 74 00243 04 115 1144)

(a) Freeze-fracture

After perilymphatic perfusion fixation with a solution containing 2% formaldehyde, 1% glutaraldehyde, 0 1 M cacodylate buffer (pH 7 3–7 4) and 25 mg% $CaCl_2$, the organ of Corti was isolated Segments 1–2 mm long were glycerinated, frozen, fractured and shadowed in a Balzers BA 360 M unit (Balzers AG, Liechtenstein) For further details the reader is referred to previous papers on the subject (Reale et al , 1975, Iurato et al , 1976)

(b) Lanthanum

The fixative used for the perilymphatic perfusion and to keep the isolated pieces of tissue overnight contained 0 7% lanthanum nitrate (Revel & Karnovsky, 1967) The same concentration of lanthanum nitrate was also contained in the washing solution (0 1 M cacodylate buffer and 0 22 M sucrose) and in the postfixative solution (1% OsO_4 solution in 0 1 M cacodylate buffer) After 2 hrs postfixation the specimens were dehydrated in graded ethanols and embedded in Epon 812 Thin sections were cut on a LKB 4800 III Ultrotome

The platinum–carbon replicas and the

sections (unstained) were mounted on Formvar–carbon membranes and observed under Siemens Elmiskop Ia and 101 electron microscopes

RESULTS

Replicas of the split plasma membrane of the supporting cells of the organ of Corti are described with the exception of those contained in the reticular membrane which have previously been investigated (Iurato et al , 1976)

We noted that the most common differentiation consists of gap junctions (Figs 1–8) As observed in other types of cells (McNutt & Weinstein, 1973, Bennett, 1973) these are composed by assemblies of 60–70 Å particles on face A and of smaller pits on the complementary face B At a higher magnification a central depression is visible on the particles of face A (Fig 4*c*)

Immediately below the zonulae occludentes of the reticular membrane, only a few small and roundish gap junctions were identified between the heads of Deiters phalanges (Fig 1*a–c*) and between the heads of the inner and outer pillars Similar small gap junctions were seen between the inner phalangeal cells and the inner pillars (Fig 2*a* *b*) as well as between Hensen s Claudius and Boettcher s cells (Fig 8*a*)

More deeply the gap junctions are exceptionally large and numerous between the foot plates of the inner pillars (Fig 3*a* *b*) of the outer pillars and between the bodies of Deiters cells (Figs 4–7) At the level of the inner pillars the gap junctions cover wide areas of the split plasma membrane delimitating areas devoid of junctional particles (Fig 3*a* *b*) Between the bodies of Deiters cells the gap junctions are generally roundish in the part towards the outer hair cells (Figs 4*a* *b* 5) but towards the basilar membrane they show the same configuration observed between the footplates of the pillars (Fig 6)

After having crossed the tunnel of Corti the nerve fibres are partially or completely surrounded by Deiters cell processes Gap junctions are visible where processes of Deiters cells are in close apposition with each other after having completely enveloped an axon in a closed channel (Fig 7*a* *b*) This aspect has already been described in thin section studies by Smith (1967) as tight junction'

We never observed gap junctions between (*a*) supporting and sensory cells (*b*) sensory cells and nerve endings and (*c*) nerve fibres Zonulae occludentes were only observed at the level of the reticular membrane (Iurato et al , 1976)

In thin sectioned specimens fixed in lanthanum containing solutions it was observed that the electron dense tracer diffuses from the perilymph through the intercellular spaces of the organ of Corti and is blocked by the zonulae occludentes of the reticular membrane Numerous gap junctions are evident between the supporting cells They display the same structure described in other epithelia (Revel & Karnovsky, 1967, McNutt & Weinstein 1973) and their location corresponds to that observed in the replicas (Fig 8*a–c*)

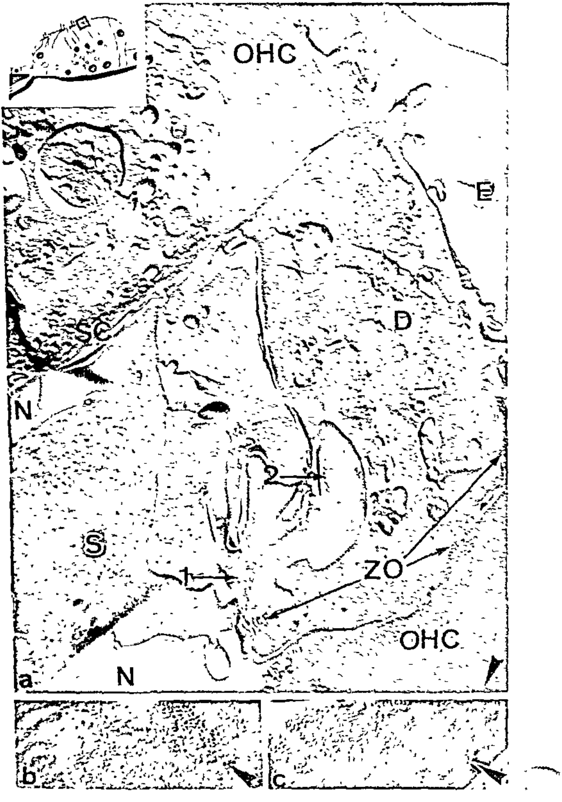
DISCUSSION

Before a clear differentiation between tight and gap junctions was introduced (Brightman & Reese, 1969), zonulae occludentes or tight

---

¹In the majority of pictures the organ of Corti is oriented with the endolymphatic surface at the top and the basilar membrane at the bottom as illustrated in Fig 9 Therefore the direction of the carbon–platinum shadowing indicated by an arrow at the right lower corner of each figure is not always oriented from bottom to top as is the general practice The schematic drawing enclosed in some tables shows the approximate location of the area illustrated in the figures

Fig 1 a–c Gap junctions between Deiters cells immediately below the zonulae occludentes of the reticular membrane *E*=endolymph *D*=Deiters cells *S* stalk of one of these cells *OHC*=outer hair cell *SC*=subsurface cisterna *ZO*=zonula occludens between Deiters cell and an outer hair cell *N*=Nuel s space The arrow 1 points to a gap junction on face A the arrow 2 to a gap junction on face B both occurring between adjacent Deiters cells ×24000 (*b*) and (*c*) show these two gap junctions at higher magnification ×84000


OHC
E
SC
D
N
2
S
1
ZO
OHC
a
N
b
c



Ax
T
IP
IPh
IPh
a
T
MV
MT
b

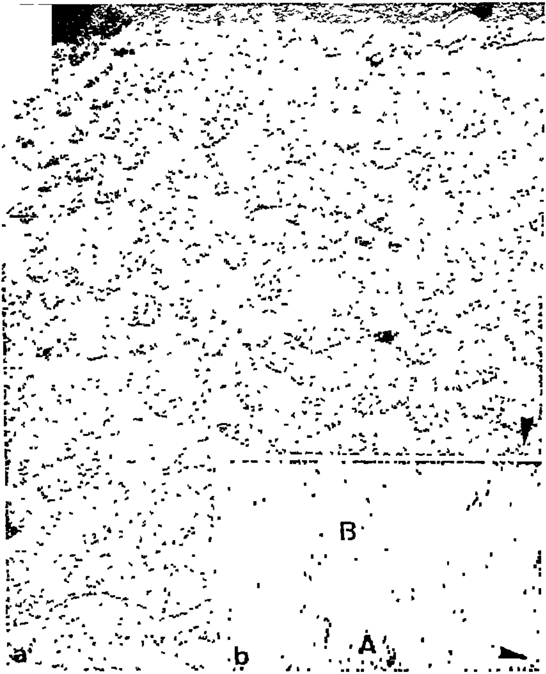


Fig 3a b Exceptionally extended gap junctions between the footplates of inner pillars In (*a*) the face A of the cleaved plasma membrane displays numerous gap junction particles surrounding smoother areas ×60000 In (*b*) particles of the same junctions are seen on face A (*A*) and pits on face B (*B*) ×90000

Fig 2a b Gap junctions between inner pillars (*IP*) and inner phalangeal cells (*IPh*) *T*–Corti tunnel *Ax*–axon ×5500 The framed area is reproduced in (*b*) at higher magnification *Mi* microvilli at the junction between two inner pillars *MT* broken microtubules of an inner pillar On the split plasma membrane of a pillar two gap junctions (*arrows*) are resolved



Fig. 4a–c. Gap junction (*arrows*) between the upper part of the body of two contiguous Deiters' cells (*D*). In (*a*) the subsurface cisternae of an outer hair cell (*OHC*) are visible ×52700. An extensive gap junction between Deiters' cells is reproduced at a higher magnification in (*b*). Face A (particles) and face B (pits) of the gap junction are resolved ×112500. (*c*) In some particles a central dot (*arrows*), possibly indicating the position of the intercellular transnexus channel (see McNutt & Weinstein, 1973), is visible ×180000.

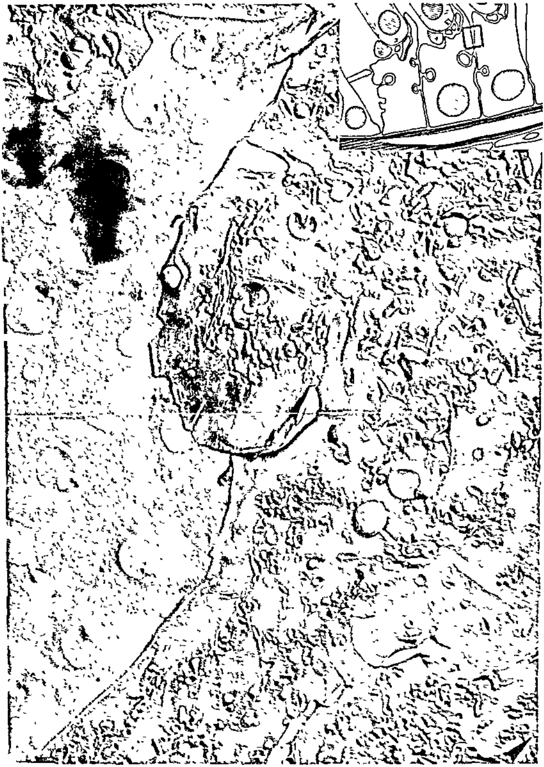


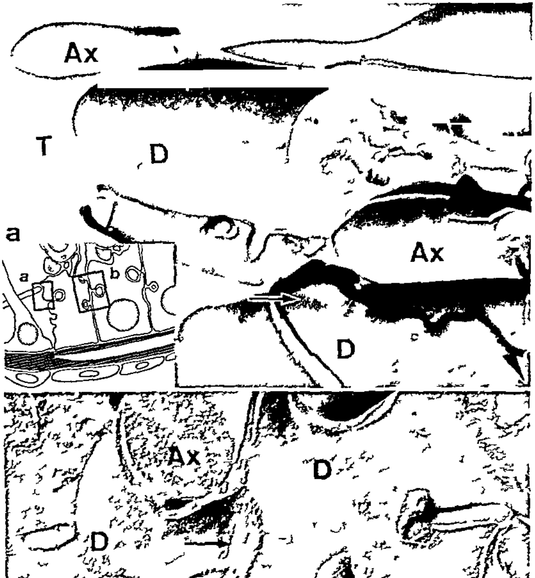
Fig 5 Gap junctions between bodies of Deiters' cells The cleavage plane runs across some Deiters' cells on the right of the picture, then within the plasma membrane of these cells on the left Here 17 roundish gap junctions can be counted ×18000



Fig 6 Gap junctions between the basal part of the body of adjacent Deiters' cells At this location the gap junctions, visible in their A and B faces, have the same aspect as between the footplates of inner pillars (see Fig 3) *BM*=basilar membrane ×96 500

junctions have been described in thin sections of the organ of Corti between the basal portions of the supporting cells (Iurato, 1967*a*, *b*) In replicas of freeze fractured specimens these zonulae appear to be gap junctions, i e maculae or spot contacts between the cells A large number of them have been found on the split plasma membrane of the supporting cells of the organ of Corti They connect reciprocally all the supporting cells (border, inner phalangeal, inner and outer pillars, Deiters', Hensen's, Claudius' and Boettcher's as well as the cells of the outer sulcus) Since no gap junctions were observed between hair cells and nerve endings, at this level the transmission should be chemically mediated, as already postulated by Davies (1959)

It is well known that gap junctions account for the movement of ions (McNutt & Weinstein, 1970), metabolites (Gilula et al , 1972) and other substances of small molecular size (Bennett, 1973, McNutt & Weinstein, 1973), from cytoplasm to cytoplasm of coupled cells without leakage into the extracellular space A variety of data indicate that gap junctions also act as electrotonic junctions in the transmission of electrical signals between excitable cells (Bennett, 1973)



F g *7a* *b* Gap junc ons (*a* ow s) between De e s cell p ocesses (*D*) wh ch surround axons (*Ax*) *T* tunnel of Cort (*a*) ×36000 (*b*) ×85000

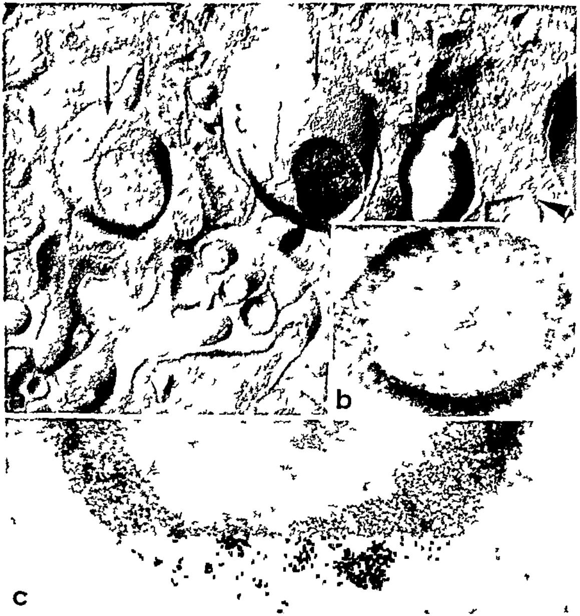


Fig 8a–c Gap junctions between Boettcher s cells in repl cas of freeze-cleaved specimens (*a*) and in th n sec t on after f xation in presence of lanthanum (*b c*) The gap junct ons are frequently located on indentations of adjacent cells (arrows in *a*) ×53 000 One of these ndentat ons has been cross cut in (*b*) ×80 000 The right part of this junct on has been reproduced at higher mag nif cat on in (*c*) Electron-dense tracer ev dences a char acterist c polygonal pattern ×??4 000

It is difficult to explain the significance of the gap junctions connecting the cytoplasm of all the supporting cells of the organ of Corti A form of equilibration or buffering tending to even out the distribution of the work per formed was suggested as function of the gap junctions between inexcitable cells by Bennett (1973) and the hypothesis could also be ap plied to our case Co operation between cells of unequal competence is also an attractive hypothesis (Bennett 1973) and it could be applied to epithelia like the organ of Corti containing supporting cells of different types

Penetrating the organ of Corti with elec

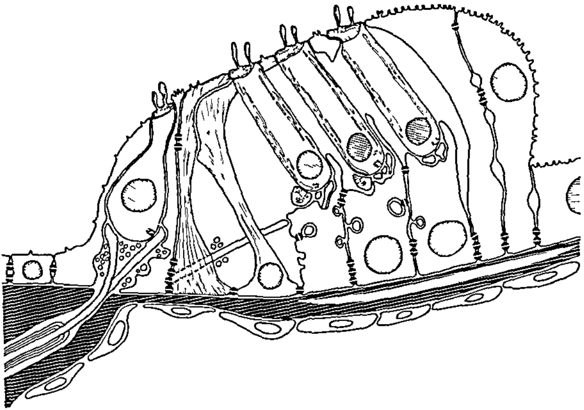


Fig 9 Schematic drawing illustrating the location of the gap junctions among the supporting cells in the organ of Corti The gap junctions are indicated by black dots crossed by a white line The line corresponds to the intercellular transnexus channel As summarized in this schema and according to the function currently attributed to the gap junctions (see McNutt & Weinstein 1973 Bennett 1973) all the supporting cells in the organ of Corti are electrotonically and metabolically coupled

trodes both Sohmer et al (1971) and Fex (1974) recorded potentials close to 0 mV for relatively long distances and time periods, in all probability corresponding to extracellular spaces Large negative potentials were also recorded inside the organ of Corti by Fex (1974) who could hold negativities for several minutes For the interpretation of these re cordings it will be kept in mind that the sup porting cells of the organ of Corti have their cytoplasms electronically and metabolically coupled by means of extensive gap junctions

Gap junctions are easily bypassed by var ious extracellular tracers (e g horseradish peroxidase Brightman & Reese 1969) and do not represent a barrier to the diffusion of substances in extracellular spaces In the organ of Corti between the perilymph of the scala tympani and the cortilymph the following structures are interposed the discontin uous layer of mesothelial cells covering the tympanic aspect of the basilar membrane the ground substance and the fibres of the basilar membrane, a basement membrane, the layer of the supporting cells which are frequently connected by gap junctions

These structures have all been shown to be permeable to or bypassed by vital stains (Tonndorf et al , 1962) and electron-dense tracers as horseradish peroxidase (Duvall & Sutherland 1972, Jahnke, 1972 1973, De Lorenzo et al 1973) and thorium dioxide (v Ilberg 1968 v Ilberg & Vosteen 1969)

After thorium dioxide administration in the

perilymph via the subarachnoid space, Angelborg (1975) found a very limited passage of tracer to the organ of Corti and concluded that there is no proof of free communication between perilymph and cortilymph

Engstrom (1960) pointed out that a strong argument against a free communication between the two fluids is the circumstance that the cells lining the cortilymph are of ecto dermal origin whereas those covering the tympanic aspect of the basilar membrane are mesothelial cells

The only structure interposed between the perilymph of the scala tympani and the cor tilymph capable to play a role in diffusion phenomena seems to be the basement mem brane lining the vestibular aspect of the basilar membrane The absence of zonulae occlu dentes between perilymph and intercellular spaces of the organ of Corti supports the idea that cortilymph and perilymph have a similar chemical composition (v Ilberg, 1968, v Il berg & Vosteen, 1969)

ACKNOWLEDGEMENT

We wish to express our thanks to Miss Carole David for her help in checking the English style of the cript and to Mr H Heidrich for his excellent inical assistance

ZUSAMMENFASSUNG

Die Zellverbindungen im Cortischen Organ wurden mit Hilfe der Gefrierbrechungsmethode am Innenohr vom Chinchilla untersucht Zwischen allen Stützzellen be sonders in deren basalem Bereich sind zahlreiche gap junctions zu finden So sind die Stutzzellen als elek trisch und metabolisch gekoppelt anzusehen Gap junctions wurden weder zwischen Stutz und Sinneszel len beobachtet noch zwischen Sinneszellen und Nerven endigungen in diesem Bereich wird die Erregungsuber tragung chemisch geschehen

REFERENCES

Angelborg C 1975 Distribution of macromolecular tracer particles (Thorotrast®) in the cochlea An electron microscopic study in guinea pig Part I The organ of Corti the basilar membrane and the tympanic covering layer *Acta Otolaryngol* (Stockh) Suppl *319* 19

Bennett M V L 1973 Function of electrotonic junctions in embryonic and adult tissues *Fed Proc 32* 65

Brightman M W & Reese T S 1969 Junctions between intimately apposed cell membranes in the vertebrate brain *J Cell Biol 40* 648

Davies H 1959 Excitation of auditory receptors In *Handbook of Physiology* Section I *Neurophysiology* (ed J Field) vol I pp 565–584 American Physiological Society Washington D C

De Lorenzo A J D Shikory D V & Cohn E I 1973 Distribution of exogenous horseradish peroxidase in perilymphatic and endolymphatic spaces of the guinea pig cochlea In *Vascular Disorders and Hearing Defects* (ed A J D De Lorenzo) pp 185–207 University Park Press Baltimore Md

Duvall A H J & Sutherland C R 1972 Cochlear transport of horseradish peroxidase *Ann Otol Rhinol Laryngol 81* 705

Engstrom H 1960 The cortilymph the third lymph of the inner ear *Acta Morphol Neerl Scand 3* 195

Fex J 1974 Neural excitatory processes in the inner ear In *Handbook of Sensory Physiology* vol V/I *Auditory System Anatomy Physiology (Ear)* (ed W D Keidel & W D Neff) pp 585–646 Springer Verlag Berlin Heidelberg New York

Gilula N B Reeves O R & Steinbach A 1972 Metabolic coupling ionic coupling and cell contacts *Nature 235* 262

von Ilberg Ch 1968 Elektronenmikroskopische Untersuchungen uber Diffusion und Resorption von Thoriumdioxyd an der Meerschweinchenschnecke *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 192* 384

von Ilberg Ch & Vosteen K H 1969 Permeability of the inner ear membranes *Acta Otolaryngol* (Stockh) *67* 165

Iurato S 1967*a* Organ of Corti In *Submicroscopic Structure of the Inner Ear* (ed S Iurato) pp 80–106 Pergamon Press Oxford

— 1967*b* Elektronenoptische Struktur der Innenohr Membranen mit Rückschlüssen auf ihre Eignung zum Stoffaustausch *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 189* 113

Iurato S Franke K D Luciano L Wermbter G Pannese E & Reale E 1976 Fracture faces of the junctional complexes in the reticular membrane of the organ of Corti *Acta Otolaryngol* (Stockh) (in press)

Jahnke K 1972 Verteilung intrathekal applizierter Peroxydase in der Meerschweinchen Cochlea *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 202* 418

— 1973 Elektronenmikroskopische Untersuchungen uber die Permeabilitatsbarrieren des Innenohres *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 204* 199

McNutt N S & Weinstein R S 1970 The ultrastructure of the nexus A correlated thin section and freeze cleave study *J Cell Biol 47* 666

— 1973 Membrane ultrastructure at mammalian intercellular junctions *Prog Biophys Mol Biol 26* 45

E Luciano L, Franke, K D, Pannese E Wermbter, G & Iurato, S 1975 Intercellular junctions in the vascular stria and spiral ligament *J Ultrastr Res 53*, 284

Revel, J P & Karnovsky, M J 1967 Hexagonal array of subunits in intercellular junctions of the mouse heart and liver *J Cell Biol 33* C7

Smith C A 1967 Innervation of the organ of Corti In *Submicroscopic Structure of the Inner Ear* (ed S Iurato) pp 107–131 Pergamon Press Oxford

Sohmer H S Peake, W T & Weiss T F 1971 Intracochlear potential recorded with micropipets I Correlations with micropipet location *J Acoust Soc Am 50* 572

Tonndorf, J Duvall A J & Reneau, J P 1962 Permeability of intracochlear membranes to various vital stains *Ann Otol Rhinol Laryngol 71* 801

S Iurato M D
Dept of Bioacoustics
University of Bari
Policlinico
I 70124 Bari
Italy

Acta Otolaryngol 82 70–81, 1976

THE CYTOCOCHLEOGRAM IN ATOXYL TREATED GUINEA PIGS

M Anniko

From the Department of Otolaryngology Karolinska Sjukhuset Stockholm and the King Gustav V Research Institute Karolinska Institutet Stockholm Sweden

(Received November 8 1975)

Abstract The earliest atoxyl induced changes in the cochlea appeared in the upper and medial parts of the 4th coil whence the changes spread progressively downwards towards the round window the extent of the changes depending on the amount of atoxyl administered and the duration of the treatment The inner hair cells were more resistant to the effects of atoxyl than the outer hair cells which were affected first The sensory cells in the 2nd and 3rd rows appeared more sensitive than the outer hair cells in the first row

Semi quantitative analysis of nuclear and ensory hair cell changes in the organ of Corti studies of ototoxic action was introduced by euman & Neubert (1958) using dissected pecimens This technique made it possible to describe precisely the localisation of damaged hair cells in the cochlea Phase contrast examination of surface preparations as well as electron microscopy following antibiotic intoxication was also reported by Engstrom et al (1964)

Beck & Krahl (1962) reported that disarrangement of the nuclei of the first row of the outer hair cells was the first sign of disarrangement of the outer hair cells in kanamycin treated guinea pigs Kohonen (1965) reported that the patterns of hair cell damage in the cytocochleogram following treatment with neomycin kanamycin or framycetin were in principle the same Ylikoski et al (1973) correlated the cytocochleogram and audiogram findings in gentamicin treated guinea pigs Ylikoski (1974) documented the relationship between the audiogram and cochleogram findings in gentamicin , neomycin and kanamycin treated guinea pigs

Recently reports have been published postulating that the hair cells of the cochlea react in a graded sequential and stereotyped fashion to insults that exceed their tolerable limit regardless of the immediate cause of injury (Hawkins 1973)

In order to investigate the hypothesis that there is a standard pattern of hair cell degeneration in the cochlea atoxyl (Pro Gen® Sodium sodium arsanilicum) which has known ototoxic effects (Miayamoto 1931 Ruedi 1951 Anniko & Wersall 1975) was used as a test substance

MATERIAL

Sixty three healthy young guinea pigs (250–350 g) with a normal Preyer s reflex without evidence of otitis media were used for the experiment The body weight of all animals the presence of the Preyer s reflex and the general behaviour of the animals were orded each day The control group con thy untreated guinea pigs

This work was supported by grants from the Karolinska Institute and the Swedish Medical Research Council (grant no 17X 720)

Table I *Table illustrating the amount of atoxyl per kg body weight administered to the guinea pigs in the experiment group*

Animal no	Atoxyl (mg/kg)	No of injections	Administered during (days)	Total dose (mg/kg)	Sacrificed after last injection (days)	Preyer reflex
1	20	47	81	940	1	+
3	20	45	78	900	1	+
4	40	46	77	1 840	1	+
6	40	45	77	1 800	1	+
12	80	8	21	640	1	−
13	80	3	4	240	5	−
16	60	9	15	540	54	−
17	60	5	9	300	3	−
18	60	10	15	600	53	−
20	70	3	2	210	1	−
22	70	4	4	280	17	(+)
24	80	1		80	2	+
26	70	1		70	1	(+)
28	70	1		70	2	−
29	70	1		70	2	−
31	70	2	1	140	20	−
32	70	1		70	24	+
33	80	1		80	24	+
34	70	1		70	24	+
39	70	2	1	140	9	−
40	70	2	2	140	24	−
42	60	1		60	31	+
43	60	1		60	31	+
44	60	1		60	6	+
45	70+60	2	5	130	29	+
48	70+30	2	5	100	29	+
50	70+30+30	3	7	130	27	+
51	60+40	2	3	100	29	+
52	70	2	1/4	140	1/4	−
53	100	1		100	1	(+)
54	70	2	1/4	140	1/4	(+)
55	140	1		140	1/2	−
57	60	5	14	300	100	+
58	60	4	14	240	100	+
59	60	5	14	300	100	(+)
63	140	1		140	1/4	−

METHODS

Administration of atoxyl

Atoxyl was administered subcutaneously as a 2% solution in sterile water The dose injected on each occasion varied between 20 and 140 mg of atoxyl per kg body-weight The total dose ranged from 60 mg to 1 840 mg of atoxyl per kg body weight The period over which atoxyl was administered varied between 1 and 81 days and the survival time before decapitation was 1 to 54 days after the last injection (Table I)

Morphological procedures

Specimens were taken for microscopy according to the technique of Wersäll (1956) After the initial perfusion for fixation with 1–2% buffered osmium tetroxide solution, the cochlea was left in this fixation fluid for 2 hours The specimen was then dehydrated in alcohol and embedded in Epon Each cochlea was split along the midline and each half coil was cut into a block with a flat surface including the whole cochlear duct The whole speci e was mounted in Epon without a cov

Table II *Table illustrating the rate of general disturbances (symptoms) and mortality rate in atoxyl treated guinea pigs in the experiment group*

Atoxyl (mg/kg)	General disturbances			Mortality rate (%)
	Weight loss	Loss of hair	General weakness	
20	(+)	(+)		0[a]
40	+	+	+	0[b]
60	+(+)	+	+	0
70	++(+)	++	++	41
80	+++	+++	+++	56
100	+++	+++	+++	–[c]
140	+++	+++	+++	–[c]
LD_{50} s c	70–80 mg/kg			

S C = subcutaneously

[a] 1 animal was suddenly lost after 46 treatments without any clinical symptoms It was not included in the mortality rate of this group

[b]

with the basilar membrane towards the glass surface of a glass slide By using the interference microscope, a complete cytocochleogram was made, recording both present and absent sensory cells (Ernstson, 1972, Ylikoski et al , 1973) Cells with smaller cytological damage as fragmentation or complete loss of the sensory hair bundles or swelling of the hair cells were counted as present From this cochleogram an average cochleogram was made by grouping five neighbouring hair cells into one group When three or more of the five hair cells were missing the group was recorded as missing, and in all other cases it was noted as present These two groups were marked in to a cochlear diagram in which the findings in each millimetre of cochlear length from the round window to the helicotrema were recorded The percentage of missing outer and inner hair cells was marked on another diagram

RESULTS

Clinical Findings

The animals were divided into two groups, according to their clinical behaviour—those without symptoms and those showing any signs of intoxication Most of the animals in the latter group showed varying degrees of loss of appetite, weight and hair and sometimes disturbances of balance were also observed (Table II)

Vestibular function was not tested but animals given a dose of 80 mg or more of atoxyl per kg body-weight at each injection often appeared to have sustained a disturbance of balance (waltzing behaviour) and a loss of the righting reflex

Weight decrease started about 6 hours after treatment, with a maximal effect 12–24 hours later Animals given a large amount of atoxyl, 60 mg or more per kg body-weight on each occasion did not regain their initial weight until several days later Smaller amounts of atoxyl, however, reduced normal weight gain during the treatment period, although the animals gained in weight to a certain extent (Diagram 1)

The smallest dose producing clinical symptoms was about 60 mg per kg body-weight on each occasion, resulting in minimal to moderate symptoms (loss of appetite and weight decrease) In surviving animals the symptoms, if any, generally lasted for a very short period

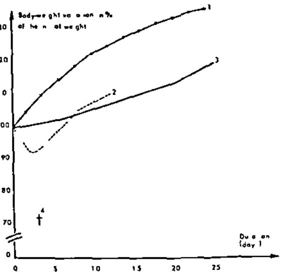


Diagram 1 Diagram demonstrating the principal body weight changes in the experiment group (atoxyl treated guinea pigs) *1* Normal weight increase *2* Animals treated with 60 mg of atoxyl per kg body weight or more at each injection *3* Long term treated guinea pigs 20–40 mg of atoxyl per kg body weight at a time *4* Animals going *ad mortem*

(12–24 hours) Loss of more than 10–15% of the initial weight during the first 24–48 hours often resulted in death

LD_{50} is between 70 and 80 mg of atoxyl per kg as a single subcutaneous injection When 19 guinea pigs were treated with 70 mg of atoxyl per kg body weight 7 animals died, whereas 80 mg of atoxyl per kg killed 5 of 9 animals

The Cytocochleogram

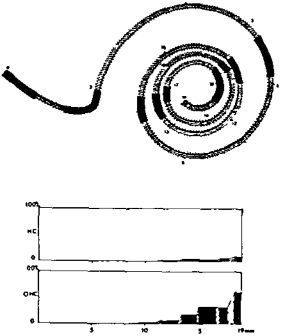
20 mg/kg

Animals belonging to this group were treated 45–47 times during 78–81 days The cytocochleograms of the animals were very similar Most of the outer hair cells of the 4th and 3rd coils were lost and only scarring remained The outer hair cells in the first row were present more frequently than those in the 2nd and 3rd rows, although many were missing in the uppermost region of the 4th turn (Fig 2) However, no acute damage could be registered

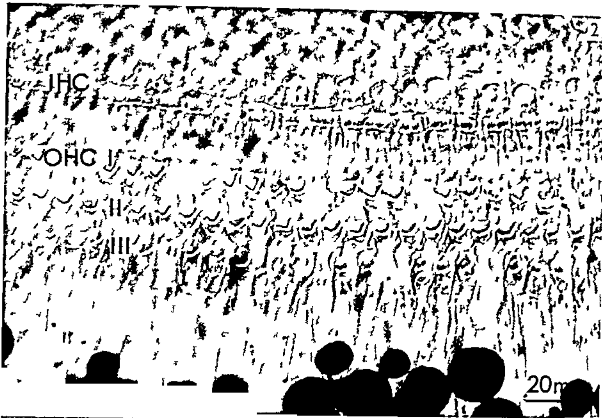
In guinea pig no 1 (Figs 1, 2) at the level of 18 8–18 3 mm from the round window 61 0% of the outer hair cells were absent, whereas only 8 1% of the inner hair cells over the same distance were missing Moving downwards from the helicotrema less damage was registered among both the outer and inner hair cells At 17 8–17 0 mm from the round window 31 4% of the outer hair cells had disappeared, at 16 7–15 1 mm 32 4%, at 14 9–13 5 mm 17 0% and at 13 1–11 5 mm 4 9% No damage could be found below the last mentioned level The inner hair cells were less affected At the 17 8–17 0 mm level 4 4% of them were missing but none were absent below 17 0 mm

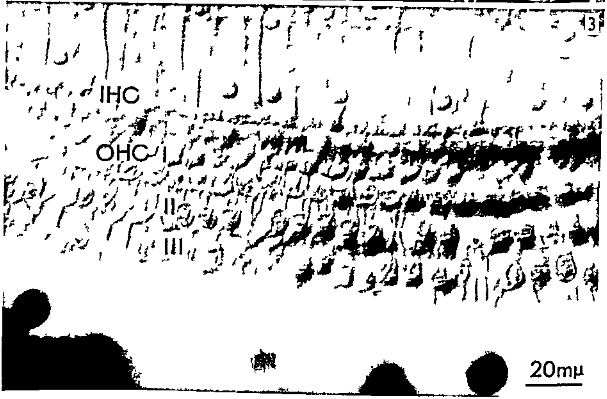
40 mg/kg

The cytocochleogram patterns were similar to those found in the group treated with 20 mg/kg on each occasion but the percentage of lost outer hair cells was higher and the damage had progressed further down In guinea pig no 4 outer hair cell losses could be registered as far down as 3 mm from the round window, where



Figs 1 4–5 8–9 and 11 Cytocochleograms illustrating the lesions of the hair cells in the cochlea. O Normal cell groups ● damaged cell groups *Diagram* Percentage of damaged cells black *Above* inner hair cells *Below* outer hair cells Fig 1 Animal No 1





F g 2 Interference contrast p cture Gu nea p g no I at the level of about 15 mm from the ound w ndow and show ng sl ght degenerat on of the outer ha r cells (*OHC I III*) ın all three rows The nner ha r cells (*IHC*) appear normal

F g 3 Interference contrast p cture Gu nea p g no 3? at the level of about 18 mm from the round w ndow The outer ha r cells ın the second and th rd rows (*OHC II III*) show severe losses No outer ha r cells in the f rst row (*OHC I*) are m ssing

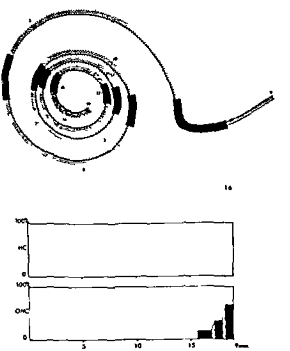


Fig 4 Cytocochleogram and hair cell degeneration diagram Animal No 16

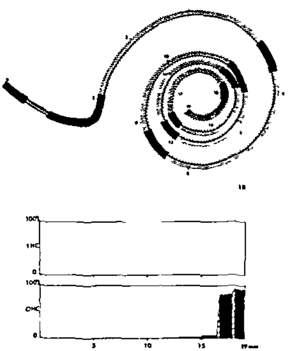


Fig 5 Cytocochleogram and hair cell degeneration diagram Animal No 18

3 3% were missing Minimal acute or subacute damage (swelling of the sensory cells) could be observed, especially in the apical coils, and fragmentation and loss of the sensory hair bundles were also seen Missing hair cells were replaced by scarring The inner hair cells were well preserved and showed only minimal scattered losses in the apical turns of about the same degree as in guinea pigs treated with 20 mg of atoxyl per kg body weight

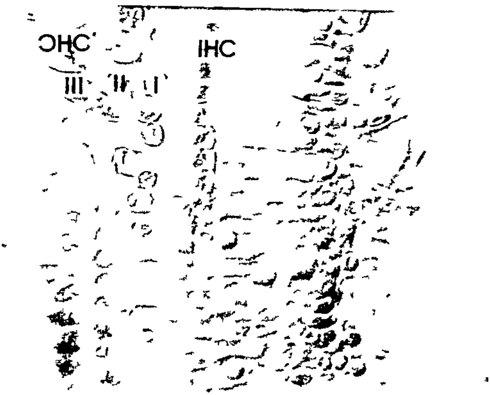
60 mg/kg

Animals receiving only one injection had similar cytocochleograms even when they were investigated after different periods of time The damage was concentrated to the second and third rows of the outer hair cells The damaged region ended rather abruptly within a distance of 10–20 neighbouring outer hair cells (" transitional zone") The damage was concentrated to the first 50–60 neighbouring sensory cells counted from the helicotrema although scattered losses were sometimes found also further down For example guinea pig no 42 had the following pattern of damage at a distance of 19 0–18 6 mm 90 4% of the outer hair cells were missing, at 18 4–17 3 mm 9 1% and at 17 0–15 8 mm 3 9% The cytocochleogram showed a normal hair cell count below the last mentioned level Some outer hair cells, especially in the third row of the 4th coil, appeared swollen and their sensory hair bundles were split up, losing their normal W form, or had even disappeared completely The inner hair cells, however, appeared completely normal

Repeated injections of atoxyl resulted in more pronounced hair cell losses (Figs 4, 5) A predominance of missing outer hair cells in the 2nd and 3rd rows was a constant finding

In guinea pig no 18 (Figs 5, 6) all outer hair cells were present at the level below 11 4 mm but at 11 4–13 0 mm, 13 3–14 9 mm, 15 1–16 5 mm, 16 7–17 9 mm and 18 1–19 0 mm levels the loss of outer hair cells was 1 3% 2 4%, 5 9%, 81 2% and 90 7% respectively The inner hair cells had a normal cell count except in the uppermost portion of the 4th coil—





F

cells (*OHC I III*) No nner hair cells (*IHC*) are m ssing however

F g 7 Interference-contrast p cture Gu nea p g no 31 at the level of about 17 5 mm from the round w ndow il lustrat ng a part of the transitional zone (swollen ha r cells and also scarring) between normal and damaged hair cell regions

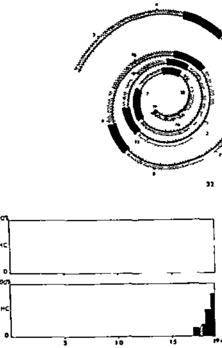


Fig 8 Cytocochleogram and hair cell degeneration diagram Animal No 32

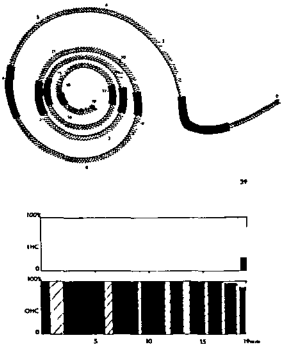


Fig 9 Cytocochleogram and hair cell degeneration diagram Animal No 39

(18 1–19 0 mm) where 1 9% were missing and had been replaced by scarring Scarring always appeared where both outer and inner hair cells had degenerated

70 mg/kg

Investigation 1 day after a single administration of atoxyl showed no pathological changes at all in the cochlea and the cytocochleogram therefore appeared completely normal If, however, the animals were studied 3 days after such treatment, slight signs of damage to the outer hair cells in the apical coil could be observed At a distance of 19 0–18 0 mm from the round window 3 9% of the outer hair cells were missing and many of those remaining appeared swollen (guinea pig no 28) The inner hair cells were unaffected Investigation 25 days after a single injection showed similar results, although an increased number of missing outer hair cells were registered at the same level and the damage could also be found further down (Fig 7) The sensory cells in the first row were more often preserved than those in the second and third rows (Fig 3)

In guinea pig no 32 (Fig 8), 80 1% of the outer hair cells were missing at a distance of 19 0–18 7 mm, 49 8% at 18 7–18 1 mm, 22 3% at 18 1–17 7 mm and 16 1% at 17 5–16 9 mm The inner hair cell count was normal

When the Preyer's reflex was compared there was a marked difference between the animals investigated 1–3 days after treatment, in which the reflex was completely lost, and the guinea pigs studied after 25 days which showed a normal reaction

Administration of more than 2×70 mg of atoxyl per kg body-weight within 24 hours always resulted in total degeneration of the outer hair cells in the whole cochlea The inner hair cells also showed severe losses in the apical coils while the basal coils were less affected (guinea pig no 39, Fig 9)

The principle for cochlear hair cell degeneration in atoxyl treatment was well illustrated by animal no 52 (2×70 mg/kg within 6 hours and investigated 12 hours after the first injection) The outer hair cells in the apical coil appeared so damaged that only cell debris remained However, about 90% of the inner



Fig 10 Interference-contrast picture Guinea pig no 13 at the level of about 10 mm from the round window All outer hair cells (*OHC I III*) are missing (replaced by scarring) while most inner hair cells (*IHC*) appear normal

air cells were still present but they showed rked swelling and disintegration of the sen ory hair bundles When moving downwards towards the round window, the 3rd turn showed an intermediate picture with minimal amounts of cell debris but different degrees of sensory cell swelling (outer and inner hair cells) while both cell types had a normal appearance in the two basal coils

Treatment with 70 mg per kg of atoxyl but with an interval of at least 24 hours between the administrations resulted in more graded damage, mainly restricted to the two apical coils

80 mg/kg

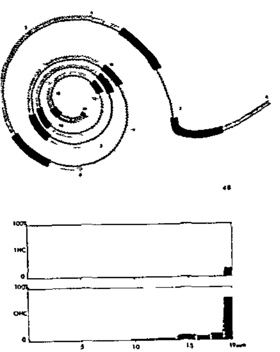
A single treatment resulted in about the same damage as observed in the group treated with 70 mg of atoxyl per kg body weight but the de generation had progressed ½–1 mm further down Repeated administration caused total degeneration of both the outer and the inner sensory cells (guinea pig no 12 Fig 10)

100 mg/kg or more

A single dose resulted in the same cytococh leogram pattern as for animals treated with 80 mg of atoxyl per kg body weight, while repeated administration caused a total degeneration of all outer and most inner hair cells with in 1–2 days

Fractionated doses

The purpose of this part of the study was to find out if it was possible to cause damage pat terns showing only minimal differences rang ing over 10–15 neighbouring outer hair cells by the administration of the same total amount of atoxyl by splitting it up into several different fractions The guinea pigs received 60+40,



ig 11 Cytocochleogram and hair cell degeneration dia ram Animal No 48

0+30, 70+30+30 and 70+60 mg of atoxyl per g body weight respectively during 3–7 days ind were investigated 27–29 days after the last idministration (guinea pig no 48, Fig 11)

The cytocochleograms were, however, rathr similar The differences in the doses injectd were thus too small to cause clear differeniation of the result

DISCUSSION

The earliest changes in the inner ears of ex erimental animals exposed to noise or treated vith ototoxic drugs have usually been seen in he outer hair cells, which are thought to be articularly sensitive to injury (Hawkins, 1973)

Degenerative changes in the cochlea usually occur at the basal end Ototoxic drugs (Duvall & Wersäll 1964, Kohonen, 1965 Lundquist & Wersall, 1966, 1967, Ylikoski 1974), acoustic overstimulation (Spoendlin, 1958, 1962, 1971, Engström & Ades, 1960, Duvall & Quick, 1969, Lim & Melnick, 1971) and the atrophic changes associated with ageing (Schuknecht, 1955, Schuknecht & Igarashi, 1964) as well as some potent diuretics (Quick & Duvall, 1970, Nakai, 1971) produce pathological changes which are most severe in the basal turn In addition, hereditary inner ear defects seem to involve the basal end more frequently than other turns of the cochlea (Kikuchi & Hilding, 1965, Anderson et al , 1968, Ernstson, 1971, 1972)

There are few reports of lesions of the hair cells in the cochlea restricted to the apical coils in the literature Alford et al (1965) reported that microscopic intravascular occlusions of the terminal branches of the labyrinthine artery caused greater changes in the apical coil of the organ of Corti than at other levels

Bernstein & Silverstein (1966) produced lesions to the apical region of the cochlea by obstructing branches of the basilar artery system in cat Kohut & Lindsay (1972) described prominent outer hair cell loss at the apex in cases with idiopathic labyrinthine hydrops

The degeneration of both the outer and the inner hair cells following atoxyl treatment start in the apical region of the cochlea The outer hair cells at the helicotrema are affected first and the damage then progresses downwards towards the round window according the extent of the changes depending on the amount of atoxyl administered and the duration of the treatment The inner hair cells appear more resistant to the effects of atoxyl Not until most of the outer hair cells are involved in the degeneration do the inner hair cells become af fected by this process

The outer hair cells of the second and third rows appear more sensitive than the hair cells in the first row This was especially so when large amounts of atoxyl were administered as a single dose resulting in higher percentage losses of the sensory cells in the 2nd and 3rd rows than in the 1st row Swelling of the sen sory cells was the first sign of degeneration, but later sensory hair bundle fragmentation

and loss as well as the formation of cell debris occurred followed by scarring

Little attempt has so far been made in the literature to explain the differential sensitivity of outer hair cells in the different rows and coils of the cochlea Beck & Krahl (1962) suggested that the slower blood flow in the basal part of the cochlea would result in locally higher concentration of the circulating drugs, and, thus, the cells there might be damaged earlier However, the systematic difference between hair cells adjacent to each other, but belonging to different rows, can scarcely be explained in this way According to Kohonen (1965) the correlation between the degree of vulnerability and differential innervation of individual outer hair cells is so good that he postulated the following hypothesis the richer the supply by large nerve endings (of a given hair cell) the more easily may its metabolism be disturbed by ototoxic drugs

Anniko & Wersall (1975) described degeneration of the stria vascularis starting at the apical region of the cochlea, suggesting that changes in the metabolism of the endolymph could be a possible cause of cochlear damage [illegible] to those observations the damaged [illegible]as, which are found in this study, thus re[illegible] the regions where the local endolymph production and reabsorption is disturbed

Kohonen (1965) searched for a systematic pattern in the distribution of cochlear sensory cell damage caused by three ototoxic drugs (neomycin, kanamycin and framycetin) He found that the pattern followed the same general principles with each of the three drugs The outer hair cells of the basal coil were affected first the damage then progressed towards the apex in the cochlea, affecting the two inner rows of the second coil and the innermost row of the two upper coils The degeneration of inner hair cells also followed a clear pattern beginning at the apex and progressing towards the base

Ylikoski (1974) reported that the earliest ultrastructural signs of degeneration, regardless of the antibiotic administered (gentamicin, kanamycin or neomycin), were observed in the outer hair cells of the basal coil The pattern of damage induced by the three antibiotics differed Neomycin seemed to attack the hook area and the apical coil first, whereas gentamicin and kanamycin usually destroyed the outer hair cells in the upper basal coil and the hook simultaneously Neomycin also displayed a clearcut affinity for the inner hair cells at the apex, cells seldom damaged after administration of gentamicin or kanamycin

Hawkins (1973) suggested that the sequence of changes in the hair cells of the cochlea appears to be much the same regardless of the immediate cause of injury, suggesting that the organ of Corti reacts in a graded, sequential and stereotyped fashion to insults that exceed the tolerable limit ("standard pattern of degeneration") The thesis of his report was that the common factor in injury to the inner ear by ageing, noise and drugs is not a direct assault on the outer hair cells but rather a breakdown of the special microhomeostatic mechanisms that govern the volume, rate of formation, and ionic composition of the inner ear fluids

Atoxyl administration results in damage to the cochlea, the primary effect being at the apical end, in contrast to most other known causes of cochlear degeneration which affect the basal end The hypothesis of a standard pattern for cochlear hair cell degeneration, regardless of the immediate cause of injury, cannot be supported However, the results of the present investigation stress the intimate relationship between the local endolymph metabolism in the stria vascularis and the organ of Corti at the adjacent level for normal cochlear function Disturbances of this homeostasis are considered to be a possible cause of the development of inner ear diseases with primary localisation at the apical end of the cochlea

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Professor Jan Wersall the Karolinska Hospital Stockholm for his guidance encouragement and critical revision of

e manuscript The skilful technical assistance of Mrs [ane Louise Spångberg and Mr Bengt Hedberg is grate lly acknowledged

ZUSAMMENFASSUNG

e fruhesten Veranderungen der Cochlea bemerkt man den oberen und mittleren Teilen der vierten Windung on der sich die Veranderungen gegen das runde Fenster erbreiten Die Ausbreitung der Veranderungen hat ihren rund in der Menge Atoxyl den man im Laufe der Be andlung den Experimenttieren zugeteilt hat Die inneren [aarzellen konnen der Wirkung des Atoxyls besser wider tehen als die außeren Haarzellen die zuerst zerstort erden Die Haarzellen der zweiten und dritten Reihe nd verletzbarer als die der ersten Reihe

REFERENCES

lford B R Shaver E F Rosenberg J J & Gu ld ford F R 1965 Physiologic and histopathologic ef fects of microembolism of the internal auditory artery *Ann Otol Rhinol Laryngol 74* 728

nderson H Henricson B Lundquist P-G Weden berg E & Wersall J 1968 Genetic hearing impair ment in Dalmatian dog *Acta Otolaryngol* (Stockh) Suppl *232* 1

\nniko M & Wersall J 1975 Damage to the stria vas cularis in the guinea pig cochlea by acute atoxyl intoxi cation *Acta Otolaryngol* (Stockh) *80* 167

3eck C & Krahl P 1962 Experimentelle und feinge webliche Untersuchungen uber die Ototoxicitat von Kanamycin *Arch Ohren Nasen Kehlkopfheilkd 179* 594

3ernstein J M & Silverstein H 1966 Anterior cerebel lar and labyrinthine arteries a study in the cat *Arch Otolaryngol 83* 422

Duvall A J & Quick C A 1969 Tracers and endo-genous debris in delineating cochlear barriers and pathways—an experimental study *Trans Am Oto-laryngol Soc 57* 21

Duvall A J & Wersäll J 1964 Site of act on of strepto-mycin upon inner ear sensory cells *Acta Otolaryngol* (Stockh) *57 581*

Engstrom H & Ades H W 1960 Effect of high-intensity noise on inner ear sensory epithelia *Acta Otolaryngol* (Stockh) Suppl *158* 219

Engstrom H Ades H W & Hawkins J E r 1964 Cytoarchitecture of the organ of Corti *Acta Oto-laryngol* (Stockh) Suppl *188 92*

Ernstson S 1971 Cochlear morphology in a strain of the waltzing guinea p g *Acta Otolaryngol* (Stockh) *71* 469

— 1972 Cochlear physiology and hair cell population in a strain of the waltzing guinea p g *Acta Otolaryngol* (Stockh) Suppl *297* 1

Hawkins J E Jr 1973 Comparative otopathology Age ing noise and ototoxic drugs *Adv Otorhinolaryngol 20* 125 Karger Basel

Kikuchi K & H ld ng D A 1965 The defective organ of Corti in Shaker I mice *Acta Otolaryngol* (Stockh) *60* 287

Kohonen A 1965 Effect of some ototoxic drugs upon the pattern and innervation of cochlear sensory cells in the guinea pig *Acta Otolaryngol* (Stockh) Suppl *208* 1

Kohut R I & Lindsay J R 1972 Pathologic changes in idiopathic labyrinthine hydrops *Acta Otolaryngol* (Stockh) *73* 402

Lim D J & Melnick W 1971 Acoustic damage of the cochlea a scanning and transmission electron micro-scopic observation *Arch Otolaryngol 94* 294

Lundquist P-G & Wersall J 1966 Kanamycin induced changes in the cochlear hair cells of the guinea pig *Z Zellforsch 72* 543

— 1967 The ototoxic effect of gentamicin—an electron microscopic study In *Gentamicin* First Interna tional Symposium pp 26–46 Schwabe & Co Basel

Miayamoto T 1931 Experimentelle Untersuchungen uber die Schadigung des Gehororgans durch die Gift wirkung der Arzneimittel *Die Wirkung des Atoxyls auf das Gehororgan* Arb aus der Medizinischen Uni versitat Okayama 2 Band 3 Heft (Japan)

Nakai Y 1971 Electron microscopic study of the inner ear after ethacrynic acid intoxication *Pract Otorhinolaryngol* (Basel) *33* 366

Neuman G & Neubert K 1958 Die Sensularien des Innenohres unter der Einwirkung von Streptomycin *Arzneim Forsch 8* 63

Quick C A & Duvall A J 1970 Early changes in the cochlear duct from ethacrynic acid an electron micro-scopic evaluation *Laryngoscope 80* 954

Rued L 1951 Some animal experimental findings on the functions of the inner ear *The Scientif c Papers of the American Otological Society LXXXVIII* 993

Schuknecht H F 1955 Presbyacusis *Laryngoscope 65* 402

Schuknecht H F & Igarashi M 1964 Pathology of slowly progressive sensorineural deafness *Trans Am Acad Ophtalmol Otolaryngol 68* 222

Spoendlin H 1958 Submikroskopische Veranderungen am Cortischen Organ des Meerschweinchens nach akustischer Belastung *Pract Otorhinolaryngol* (Basel) *71* 657

— 1962 Ultrastructure of the organ of Corti in normal and acoustically stimulated animals *Ann Otol Rhinol Laryngol 71* 657

— 1971 Primary structural changes in the organ of Corti after acoustic overstimulation *Acta Otolaryngol* (Stockh) *71* 166

Wersäll J 1956 Stud es on the structure and innervation of the cristae ampullares in the guinea p g *Acta Oto-laryngol* (Stockh) Suppl *126* 1

Ylikoski J Wersäll J & Björkroth B 1973 Hearing loss and cochlear pathology in gentamicin intoxicated guinea pigs *Acta Pathol Microbiol Scand* (B) *81* Suppl *241* 30

Yl koski J in cooperat on with Jan Wersäll and B rgitta Björkroth 1974 Correlative stud es on the cochlear pathology and hearing loss in guinea p gs after intoxica tion with ototoxic ant biotics *Acta Otolaryngol* (Stockh) Suppl *326* 1

M Ann ko M D
King Gustav V Research Inst tute
S 104 01 Stockholm 60 Sweden

Acta Otolaryngol 82 82–94, 1976

NEURAL DAMAGE IN THE GUINEA PIG COCHLEA AFTER NOISE EXPOSURE

A Light Microscopic Study

Ch G Wright

From the Kresge Hearing Research Institute Department of Otorhinolaryngology University of Michigan Medical School Ann Arbor Mich USA

(Received September 29, 1975)

Abstract Neural damage produced by exposure to 8 kHz octave band noise was studied by light microscopy in guinea pigs Abnormal nerve endings were found beneath both inner and outer hair cells within 24 hours after exposure at either 118 or 120 dB SPL In areas where the organ of Corti was destroyed degeneration of myelinated nerve fibers was seen immediately after 24-hour exposures Nerve degeneration secondary to hair cell loss was observed However degeneration of myelinated fibers occurred in some cases without significant hair cell loss At survival times as long as two months nerve fibers coursing near capillaries were still present in extensively damaged areas In three animals nerve fibers which had apparently regenerated into regions where the sensory epithelium was destroyed were found distal to the habenula perforata Evidence of regenerative activity was also noted in the osseous spiral lamina

Staining methods for the demonstration of unmyelinated nerve fibers by light microscopy have recently been used in our laboratory for study of the cochlear nerve supply (Wright & Preston, 1973, Wright, 1975, Wright & Preston, 1975) In combination with the surface preparation or whole-mount technique (Engström et al , 1964) these stains provide an overview of the peripheral innervation which is difficult or impossible to obtain by electron microscopy In such preparations nerve fibers can be traced over long distances with relative ease and abnormal or degenerating neural elements can readily be recognized

We felt that this approach might serve as a useful adjunct to electron microscopy in the investigation of neural damage resulting from noise exposure Therefore, the present study was undertaken to determine what neuropathological changes are observable by light microscopy in cochlear tissue from noise-exposed animals Both the zinc iodide osmic acid and silver nitrate stains were applied to whole mount preparations of the organ of Corti from guinea pigs exposed to octave-band noise The following report is a survey of our findings in this material

METHODS

Altogether, 17 guinea pigs weighing 200-300 g were exposed to an 8 kHz octave-band of noise Nine of these animals (Group I) were exposed at 118 dB SPL, 8 received exposures at 120 dB (Group II) All animals in Group I received a single noise exposure, 24 hours in duration, and were killed either immediately afterward or at survival times of 1 day to 2 weeks The animals of Group II received single exposures of 4 to 24 hours and were permitted survival times of up to 2 months

The exact post-exposure survival times

'le I

oup I 118 dB 24 h		Group II 120 dB		
ost-exposure ival time	No of animals	Duration of exposure (h)	No of animals	Post-exposure survival time
led immediately	2	4	3	Killed immediately, 1 day 2 days
1 day	1	6	3	Killed immediately, 2 days, 5 weeks
1 week	3	8	1	2 months
2 weeks	3	24	1	6 weeks

ed each animal are given in Table I, each ˆ the survival times listed under Group II ..ents one animal

All animals were noise exposed in an IAC ˙ le-walled sound chamber During the nosures they were individually housed in wire cages placed directly beneath the dspeaker, which was positioned approxi- 'y 18 inches above the animals' heads

Fifteen ears from Group I and 13 ears from Group II were studied by light microscopy. (The remaining 6 were prepared for electron .. croscopy) The inner ear tissues were fixed d stained according to methods for either zinc iodide osmic acid (ZIO) or silver staining as described in our previous publications (Wright & Preston, 1973, Wright, 1975) Whole-mount specimens of the organ of Corti were then examined by brightfield light microscopy The experimental material was compared with a large collection of normal cochlear tissue prepared and stained by the same methods as were used for the noise-exposed ears

RESULTS

In both groups of animals included in this study there was considerable variability in the degree of damage to the organ of Corti caused by noise exposure The extent and localiza tion of hair cell loss in these animals will not be described in detail in this report However, before turning to a specific consideration of neural changes, some general comments should be made concerning the sensory and supporting cell damage that was observed

Eight of the 15 ears studied from Group I had single, discrete lesions of the organ of Corti in which the normal cytoarchitecture was destroyed These sharply-delineated "focal" lesions were located in the mid-region of the basal turn and ranged in length from about 0 2 mm to 3 mm The lesions were usually found to be of different lengths in the two ears of a single animal and often only one ear showed a focal lesion When such lesions were present in animals with survival times of several days or more there was moderate to severe outer hair cell loss on either side of the severely damaged area and scattered hair cell loss in the upper cochlear turns The other 7 ears included in Group I which did not have sharply defined lesions showed scattered outer hair cell and pillar cell loss in all turns, there were no well-defined areas of maximum damage

In Group II the effects of noise exposure were quite similar However, when focal lesions occurred they tended to be shorter in length (0 15 to 0 6 mm) and were sometimes multiple That is, in several cases two separate lesions approximately 0 2 mm long were present in the basal turn Otherwise, scattered hair cell loss like that seen in Group I was found Much more widespread damage was observed in the one animal exposed to 120 dB for 24 hours In this case there were large areas of total destruction of the sensory epithelium in all cochlear turns

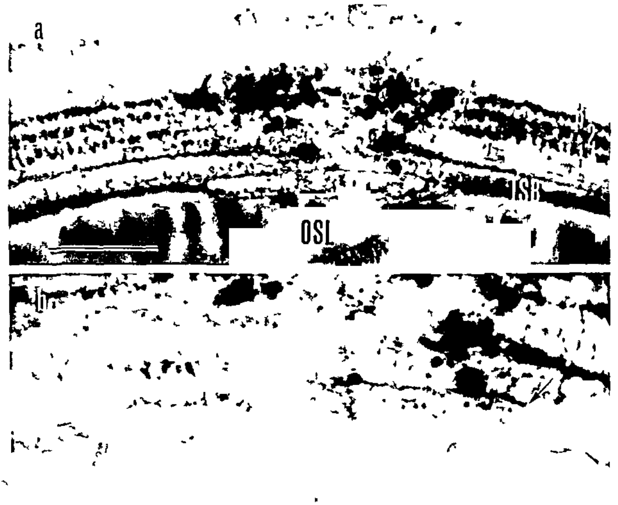


Fig 1 (*a*) Focal lesion in the basal turn of the organ of Corti from a guinea pig killed immediately after a 120 dB 6-hour exposure (*b*) Higher power view of inner spiral bundle (*ISB*) in which large vacuoles can be seen (arrows) ZIO-stained specimen Bars at lower left represent 100 microns *OSL* osseous spiral lamina 1-2-3 nerve endings beneath the three rows of outer hair cells

Neural changes seen within 24 hours post exposure

A total of 10 ears from 6 animals exposed to noise at either 118 or 120 dB and killed within 24 hours after the end of the exposure were studied In 6 of these ears there were one or more areas of severe focal damage to the organ of Corti

In the region included in each lesion the normal cellular pattern was destroyed No outer hair cells were recognizable and the row of inner hair cells was either badly disrupted or missing entirely No nerve endings or fibers remained in the area of the outer hair cells and tunnel crossing fibers were not seen Some fragments of the spiral tunnel bundle were usually still present In all cases the inner spiral nerve bundle was present but appeared to be split apart longitudinally and contained large clear vacuoles as shown in Figs 1 and 3

Clear spaces very similar in appearance to the vacuoles seen in the region of the inner spiral bundle were occasionally found directly beneath the inner hair cells in portions of

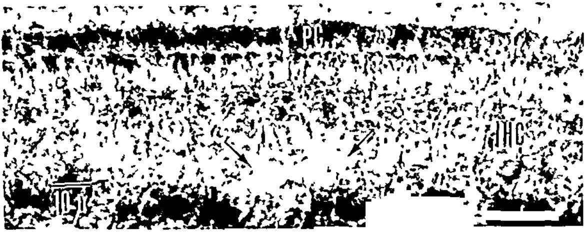


Fig 2 Vacuoles (arrows) beneath inner hair cells (*IHC*) in the third cochlear turn of an animal killed immediately after a 120 dB 6-hour exposure ZIO stain *PC* head plates of pillar cells

the organ of Corti far removed from severe lesions For example, Fig 2 illustrates vacuoles under inner hair cells in the third cochlear turn of an animal killed immediately after a 6-hour exposure at 120 dB These spaces were observed only in animals which received short duration exposures (4 to 6 hours) and which had short post exposure survival times

In 2 ears from different animals, both of which were killed immediately after 24-hour exposures at 118 dB, focal lesions were found in the basal turn Myelinated nerve fibers approaching these damaged areas were clearly fragmented and in the early stages of degeneration However, the fragmentation was confined to a narrow region near the habenula perforata



Fig 3 Darkly stained nerve endings (large arrow) adjacent to a focal lesion in the basal turn 120 dB 4-hour exposure 24-hour post exposure survival time Small arrow indicates a large vacuole beneath inner hair cells (*IHC*) ZIO stain 1 2 3 rows of nerve endings under outer hair cells

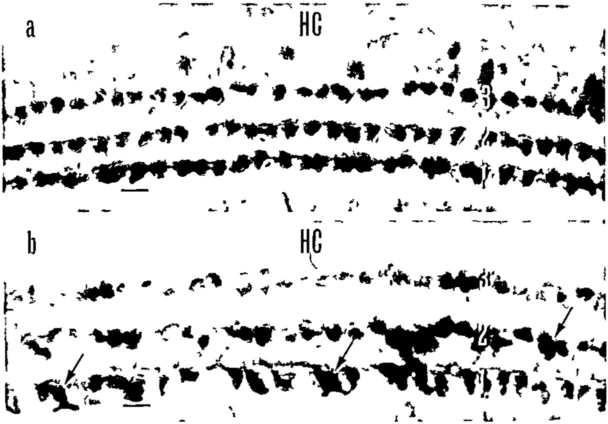


Fig 4 (*a*) Nerve endings beneath the three rows (1 2 3) of outer hair cells in upper basal turn of a normal guinea pig cochlea (*b*) Swollen dense nerve endings (arrows) in upper basal turn from an animal killed immed ately after a 120 dB 6-hour exposure ZIO stain Bars at lower left represent 10 microns *HC* Hensen cells

Particularly with short duration exposures and brief survival times the boundaries of focal lesions were sharp and distinct Immediately adjacent to the region of total disruption the reticular lamina often appeared quite normal with no hair cells missing However, in these areas bordering lesions the efferent nerve endings beneath the outer hair cells were sometimes impregnated by the ZIO stain in a peculiarly dark dense fashion An example of this type of staining reaction is shown in Fig 3 Abnormally dark efferent terminals on outer hair cells were also found in other portions of the basal and lower second turns well removed from focal lesions In such areas there was little or no loss of outer hair cells, but the clusters of efferent nerve endings were stained with unusual density and they often seemed to be swollen or misshapen, as illustrated in Fig 4 In one case darkly stained swollen efferent endings were present in the basal turn of a cochlea in which no focal lesion occurred and only minor hair cell loss was found following a 4 hour exposure at 120 dB These dense, darkly stained nerve endings were not seen in animals with post exposure survival times of more than 24 hours

Neural changes seen within two to seven days post exposure

A total of 8 ears from 5 animals exposed at either 118 or 120 dB and killed within one week after the exposure were studied by light microscopy In general, a greater hair cell loss was noted in these animals compared to those killed within 24 hours after exposure

Two animals with one-week survival times



Fig 5 (*a*) Reticular lamina showing severe loss of sensory cells in outer hair cell region (*OHC*) specimen from basal turn of an animal killed 7 days after a 118 dB 24-hour exposure *PC* pillar cells (*b*) Deeper level of focus showing swelling and coalescence of neural elements (arrows) after loss of hair cells *HC* Hensen cells Bars at lower left represent 10 microns ZIO stain

after 24 hour exposures at 118 dB had massive outer hair cell loss in the basal and second cochlear turns In the areas where the outer hair cell loss was virtually complete the nerve fibers and endings peripheral to the tunnel of Corti were clearly in the process of degeneration Breakdown and coalescence of neural material in the outer hair cell region of one of these animals is illustrated in Fig 5

Where only scattered hair cells were missing a patchy degeneration of nerve endings was found to accompany the loss of receptor cells An example of the neat correspondence between hair cell and nerve degeneration in such areas is shown in Fig 6

After one week degeneration of myelinated fibers was well advanced in regions in which the organ of Corti had been completely destroyed This is illustrated in Fig 7 which shows degeneration of myelinated fibers extending well into the osseous spiral lamina in an animal killed 7 days after a 24 hour exposure at 118 dB

An unexpected and dramatic degeneration of myelinated nerve fibers in the extreme apical turn was found in two noise exposed animals in this group In one animal the apical degeneration occurred in both ears, it received a 4 hour, 120-dB exposure and had a 2-day survival time The other guinea pig which survived one week after a 24-hour, 118-dB exposure, showed degeneration of apical myelinated fibers only in one ear In each of these cases little or no damage to the organ of Corti itself was evident There was no loss of inner hair cells or pillar cells and

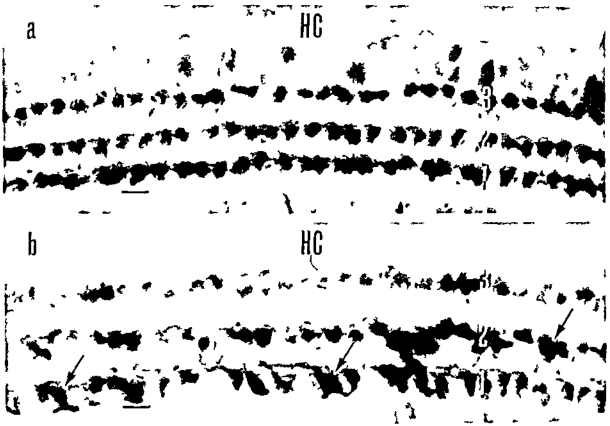


Fig 4 (*a*) Nerve end ngs beneath the three rows (1 2 3) of outer hair cells in upper basal turn of a normal guinea pig cochlea (*b*) Swollen dense nerve endings (arrows) in upper basal turn from an animal killed immed ately after a 120 dB 6-hour exposure ZIO sta n Bars at lower left represent 10 microns *HC* Hensen cells

Particularly with short duration exposures and brief survival times the boundaries of focal lesions were sharp and distinct Immediately adjacent to the region of total disruption the reticular lamina often appeared quite normal with no hair cells missing However in these areas bordering lesions the efferent nerve endings beneath the outer hair cells were sometimes impregnated by the ZIO stain in a peculiarly dark dense fashion An example of this type of staining reaction is shown in Fig 3 Abnormally dark efferent terminals on outer hair cells were also found in other portions of the basal and lower second turns well removed from focal lesions In such areas there was little or no loss of outer hair cells but the clusters of efferent nerve endings were stained with unusual density and they often seemed to be swollen or misshapen as illustrated in Fig 4 In one case darkly stained swollen efferent endings were present in the basal turn of a cochlea in which no focal lesion occurred and only minor hair cell loss was found following a 4 hour exposure at 120 dB These dense darkly stained nerve endings were not seen in animals with post exposure survival times of more than 24 hours

Neural changes seen within two to seven days post exposure

A total of 8 ears from 5 animals exposed at either 118 or 120 dB and killed within one week after the exposure were studied by light microscopy In general a greater hair cell loss was noted in these animals compared to those killed within 24 hours after exposure

Two animals with one week survival times



Fig 7 Massive lesion (left hand portion of micrograph) in basal turn showing total destruction of organ of Corti and degeneration of myelinated nerve fibers in osseous spiral lamina (*OSL*) 7 days after a 118 dB 24-hour exposure *BM* basilar membrane *MF* myelinated nerve fibers remaining in region where organ of Corti is still present ZIO stain

large diameter and could easily be traced straight radially directly into the osseous spiral lamina The disorganized meandering of these fibers peripheral to the osseous lamina is illustrated in Fig 9 It should be noted that the *terminal course of these fibers is wholly uncharacteristic* of the normal pattern of distribution of nerve fibers inside the organ of Corti The large size of the fibers suggests that they might be myelinated However, this could not be determined with certainty in the silver stained preparations in which they were seen

Within the osseous spiral lamina in regions where much neural degeneration had occurred myelinated fibers were observed which appeared to be looping back upon themselves as shown in Fig 9 The course of such fibers suggests that they might have been growing toward the habenula perforata only to turn back in the direction of the modiolus before reaching the basilar membrane

Finally in areas devoid of sensory epithelium it was occasionally possible to observe tiny, beaded nerve fibers coursing near blood vessels peripheral to the habenula perforata These fibers were stained lightly by both the ZIO and silver stains However, they could sometimes be traced from within the osseous *lamina outward to the basilar membrane usually* accompanying capillaries Such fibers were seen in animals killed as long as 2 months post exposure, as shown in Fig 10

DISCUSSION

The present study demonstrates that a variety of structural alterations in the cochlear nerve supply are observable by light microscopy following noise exposure

Several of the neural changes seen in this investigation can be readily correlated with ultrastructural alterations produced by acoustic trauma which recently have been described by Spoendlin and other workers For example Spoendlin (1971) found an increased density of packing of synaptic vesicles

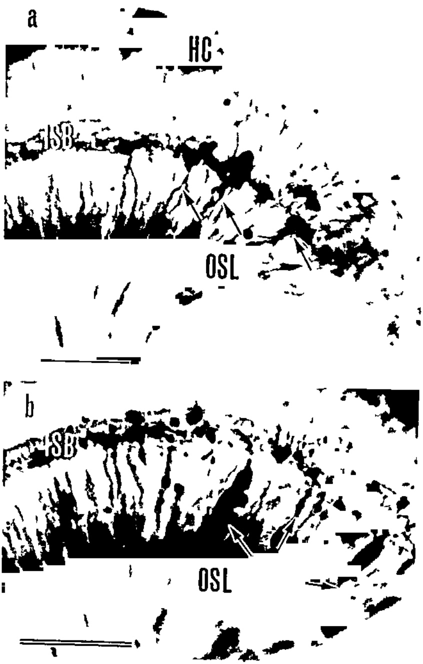


Fig 8 (*a*) Nerve degeneration (arrows) extending into osseous spiral lamina (*OSL*) in extreme apical turn 2 days after a 120 dB 4 hour exposure (*b*) Similar degeneration of myelinated fibers (arrows) in apical turn of an animal killed 7 days after a 118 dB 24-hour exposure Bars at lower left represent 100 microns *ISB* inner spiral bundle *HC* Hensen cells ZIO stain

and abnormally dense ground substance in efferent nerve terminals after noise exposures of one hour at 130 dB This phenomenon probably accounts for the dark staining reaction and distorted appearance of efferent endings on outer hair cells which we observed in animals with short post exposure survival times It should be noted that these dense swollen nerve endings were seen before any degeneration of the hair cells themselves was observable In an earlier electron microscopic study Engstrom & Ades (1960) also found swollen nerve terminals at the bases of hair cells after short-duration exposures to jet engine noise

The large clear spaces seen beneath the inner hair cells apparently correspond to the greatly enlarged afferent nerve endings Spoendlin (1969 1971) has observed after



Fig. 9. (*a*) Regenerated nerve fibers (arrows) immediately above basilar membrane (*BM*) in an area in which the organ of Corti was totally destroyed. 118 dB. 24-hour exposure. 2 weeks survival time. (*b*) Myelinated nerve fibers (arrows) forming loops in osseous spiral lamina (*OSL*) adjacent to a severe lesion of the organ of Corti. 120 dB. 8-hour exposure. 2 months survival time. Silver stained specimens.

both noise exposure and ischemia. More recently Ades et al. (1974) demonstrated a similar swelling of neural elements in the inner spiral bundle region of chinchillas exposed to noise. Also Lim & Melnick (1971) noted enlarged mitochondria in afferent terminals on inner hair cells of the guinea pig after noise exposures lasting 4 to 24 hours.

In areas of severe damage the afferent dendrites almost certainly rupture, and degeneration of the myelinated portions of the nerve fibers quickly commences, as evidenced by



Fig 8 (*a*) Nerve degeneration (arrows) extending into osseous spiral lamina (*OSL*) in extreme apical turn 2 days after a 120 dB 4 hour exposure (*b*) Similar degeneration of myelinated fibers (arrows) in apical turn of an animal killed 7 days after a 118 dB 24-hour exposure Bars at lower left represent 100 microns *ISB* inner spiral bundle *HC* Hensen cells ZIO stain

and abnormally dense ground substance in efferent nerve terminals after noise exposures of one hour at 130 dB This phenomenon probably accounts for the dark staining reaction and distorted appearance of efferent endings on outer hair cells which we observed in animals with short post exposure survival times It should be noted that these dense, swollen nerve endings were seen before any degeneration of the hair cells themselves was observable In an earlier electron microscopic study, Engstrom & Ades (1960) also found swollen nerve terminals at the bases of hair cells after short-duration exposures to jet engine noise

The large, clear spaces seen beneath the inner hair cells apparently correspond to the greatly enlarged afferent nerve endings Spoendlin (1969, 1971) has observed after

similar to that observed by Engstrom et al 1966) in guinea pigs treated with ototoxic rugs Also, the patchy degeneration of nerve ndings and outer hair cells found in less everely damaged areas appears identical to e degeneration Engstrom et al have de-cribed in animals given ototoxic drugs or xposed to impulse noise produced by small-aliber fire arms

In their study of ototoxicity, Engström t al (1966) demonstrated the presence of very in, presumably autonomic nerve fibers in the uinea pig cochlea which resist damage by rug treatment Delicate, beaded nerve fibers ere observed in the present study which ac ompanied capillaries in the osseous spiral amina and under the basilar membrane in ireas of total destruction of the organ of Corti uch fibers were seen in material from animals illed as long as two months post exposure hese nerve fibers were also observable in iormal ears and have been noted previously in uinea pigs by Hawkins (1973) However, ifter degeneration of the sensory epithelium ind myelinated nerve fibers they are easier to bserve with the light microscope We are in greement with Engstrom et al that such ibers are autonomic and that they persist after estruction of the organ of Corti and mye inated nerve supply

A finding of the present study which de-erves further investigation is the apparent re eneration of nerve fibers into badly damaged egions The disorganized, tangled course of the nerve fibers seen near the basilar mem-rane is so unlike the normal pattern of nerve istribution that it seems unlikely that they are merely survivors of the acoustic trauma which ailed to degenerate Also the large diameter of these fibers indicates that they may be mye-inated in a position well distal to the habenula perforata In addition the peculiar hairpin loops formed by myelinated fibers in regions of extensive degeneration are suggestive of re-generative activity within the osseous spiral lamina It should be recalled that Johnsson & Hawkins (1972) have described occasional myelinated nerve fibers wandering along the basilar membrane in portions of the cat coch-lea damaged by gentamicin treatment and in Dalmatian dogs with hereditary deafness In rare cases, Johnsson (unpublished observa-tions) has seen such fibers in human material as well These observations, therefore, indi-cate that the peripheral processes of cochlear ganglion cells do possess some capacity for re-generation following injury

ACKNOWLEDGEMENT

The author is indebted to Prof Joseph E Hawkins jr for valuable advice throughout this study and for construc tive criticism of the manuscript

The assistance of Verne T Maulbetsch James W Bruce and Robert L Combs is also gratefully ac knowledged

ZUSAMMENFASSUNG

Es wurde eine lichtmikroskopische Untersuchung der Nervenschädigung in der Meerschweinchenschnecke nach 4 bis 24stündiger Beschallung (8 kHz Oktavband 118–120 dB SPL) durchgeführt In Tieren die innerhalb von 24 Stunden nach Lärmeinwirkung getötet wurden konnten anomale Nervenenden unterhalb der inneren und außern Haarzellen gefunden werden Degeneration von myelinisierten Nervenfasern konnte sofort nach 24stun diger Beschallung beobachtet werden Obwohl Nervende-generation im Gefolge von Haarzellverlust mehrere Tag nach Beschallung gefunden wurde trat Degeneration von myelinisierten Fasern in einigen Fällen ohne bemerkens werten Haarzellverlust auf In schwer geschädigten Re-gionen waren nach bis zu zwei Monaten noch Nervenfa sern in der Nahe von Kapillaren vorhanden In drei Tieren wurden distal der Habenula perforata Nerven fasern gefunden die anscheinend in Regionen regeneriert hatten in denen das Sinnesepithel zerstort worden war Hinweise auf regenerative Aktivitat wurden auch in der lamina spiralis ossea beobachtet

REFERENCES

Ades H W Trahiotis C & Kokko-Cunningham A 1974 Comparison of hearing thresholds and mor phological changes in the chinchilla after exposure to 4 kHz tones *Acta Otolaryngol* (Stockh) *78* 192

Engström H & Ades H W 1960 Effect of high inten sity noise on inner ear sensory epithelia. *Acta Oto-laryngol* (Stockh) Suppl *158* 219

Engström H Ades H W & Andersson A 1966 *Structural pattern of the organ of Corti* Almqvist & Wiksell Stockholm

Engstrom H Ades H W & Hawkins J E jr 1964

Cytoarchitecture of the organ of Corti *Acta Otolaryngol* (Stockh) Suppl *188* 92
Hawkins J E jr 1973 Summary (pp 148–166) In *Vascular Disorders and Hearing Defects* (ed A J Darin de Lorenzo) Univ Park Press Baltimore
Johnsson L-G & Hawkins J E jr 1972 Strial atrophy in clinical and experimental deafness *Laryngoscope 82* 1105
Lim D J & Melnick W 1971 Acoustic damage of the cochlea *Arch Otolaryngol 94* 294
Lurie M H Davies H & Hawkins J E jr 1944 Acoustic trauma of the organ of Corti in the guinea pig *Laryngoscope 54* 375
Spoendlin H 1969 Das ischamische Syndrom des Innenohres *Pract Otorhinolaryngol* (Basel) *31* 257
— 1971 Primary structural changes in the organ of Corti after acoustic overstimulation *Acta Otolaryngol* (Stockh) *71* 166
Stockwell C W Ades H W & Engström H 1969 Patterns of hair cell damage after intense auditory stimulation *Ann Otol Rhinol Laryngol 78* 1144
Wright C G 1975 Cochlear innervation in the guinea pig I The inner spiral bundle region *Acta Otolaryngol* (Stockh) *80* 2?0
Wright C G & Preston R E 1973 degeneration and distribution of efferent nerve fibers in the guinea pig organ of Corti A light and scanning electron microscopic study *Brain Res 58* 37
Wright C G & Preston R E 1975 Cochlear innervation in the guinea pig II The spiral tunnel bundle *Acta Otolaryngol* (Stockh) *80* 335

Ch G Wright Ph D
Dept of Otorhinolaryngology
Kresge Hearing Research Institute
University of Michigan Medical School
Ann Arbor Mich 48109 USA

Acta Otolaryngol 82 95–98, 1976

NEUE TIEREXPERIMENTE ZUM AEROBEN UND ANAEROBEN INNENOHRSTOFFWECHSEL

H Jakobı, P Lotz, K -D Kuhl und E -J Haberland

Aus der Klinik und Poliklinik für Hals Nasen und Ohren krankheiten der Martin Luther Universitat Halle-Wittenberg DDR

(Eingegangen am 20 September, 1975)

bstrakt Die Innenohrfunktion ist an eine standige auerstoffzufuhr gebunden Ein Beweis dafür ist die DH Untergruppenverteilung im Innenohr die an acht Arten untersucht wurde Ein glykolytischer Energieumatz im Innenohr laßt sich postmortal bei den Arten nach veisen die uber Glykogenvorräte im Innenohr verfügen ie MP_2 sind durch Glucose und Jodacetat Perfusion der calen beeinflußbar Bei Sauerstoffzufuhr werden die MP lurch Jodacetat nicht erniedrigt

ie Umsetzung mechanischer in elektrische nergie im Innenohr erfolgt unter zusätzlicher nergieheterung aus biochemischen Prozesen Es bestehen heute kaum noch Zweifel, aß diese Stoffwechselenergie gang uber viegend aus der aeroben Glukoseveratmung eliefert wird Dafur sprechen

1 die jedem Kliniker bekannten Innenohrausfalle, die auf mangelnde Durchblutung und damit auf mangelnde Sauerstoffzufuhr zu ruckzufuhren sind (perinatale Schaden, Gefaßerkrankungen, Asphyxien usw),
 das steile Absinken des endolymphatischen Ruhepotentials und des Mikrophonpotentials im Tierversuch unter Anoxie, die nach baldiger Wiederbeatmung schnell auf ihre normale Höhe ansteigen (Davis 1955, Konishi et al , 1961, 1967, Thalmann, et al , 1963, Deutsch, 1964 Tsunoo & Perlmann, 1964, Lawrence & Arbor, 1965, Honrubia et al , 1965, Faltynek & Vesely, 1967),
 die solide grundausstattung der Stria vascularis und des Cc [illegible] chondrien und Enzymen der Atmungskette (Smith, 1958, Wersäll et al , 1961, weitere Literatur bei Schätzle, 1971),
4 die aus der Isoenzymverteilung der Lactatdehydrogenase berechneten sogenannten H-Untereinheiten, die in den genannten Innenohrstrukturen weit über der 50%-Grenze liegen (Lotz et al , 1971, 1973)

Eine eventuelle Beteiligung der anaeroben Glykolyse an den energieliefernden Prozessen der Schalltransformation ist bis jetzt aber noch weitgehend ungeklart Fur eine Beteiligung der Glykolyse sprechen

1 Stria vascularis und Corti Organ sind gut mit glykolytischen Enzymen ausgestattet Diese Aussage bezieht sich allerdings nur auf Meerschweinchenversuche (Vosteen 1961, Thalmann et al , 1970),
2 bei einigen Arten, z B Meerschweinchen, Ratte, Kanguruh Ratte, Kaninchen, wurden hohe Glykogenvorrate in der Stria vascularis und dem Cortischen Organ gefunden (Vosteen, 1964, Vinnikov & Titova, 1962, Falbe-Hansen, 1963, Schätzle, 1971, Stack & Webster, 1971, Ishii et al , 1969, Thalmann, 1973),
3 das postmortale Mikrophonpotential, MP_2, ist in den ersten 30 Min stoffwechselabhängig

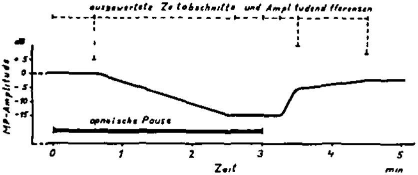


Abb 1 Mikrophonpotentiale des Meerschweinchens bei apnoischer Pause

Die Zeit 0 bedeutet beginnende Anoxie oder Exitus, der Kurvenverlauf ist in beiden Fallen identisch Der Exitus bei experimenteller Anoxie zeigt kein signifikantes Merkmal Unter Einfluß einer perilymphatischen Glukoseperfusion (Na^+ 148 mM, K^+ 5,8 mM, Cl^- 5,8 mM, SO_4^{--} 74 mM, Glukose 74 mM) bleibt das MP_2 langer stabil bzw der Abfall wird verzogert Perfusion mit Jodacetat (Na^+ 148 mM, K^+ 5,8 mM, Cl^- 144 mM, Jodessigsaure 10 mM), einem Glykolyseinhibitor, beschleunigt dagegen den Abfall des MP_2

Die Versuche zeigen, daß bei vollstandiger Anoxie oder nach dem Exitus beim Meerschweinchen das MP_2 etwa 20 Min lang anaerob, d h glykolytisch erhalten bleibt Der Befund steht nicht im Widerspruch zu den Angaben, daß das MP_2 rein physikalischen Ursprungs sei, weil der Q10-Quotient auf etwa 1 absinkt und das MPY_2 keine Sattigung zeigt Diese Beobachtungen gelten nur fur eine MP_2 Registrierung 1 Std oder langer post mortem (Low, 1973)

Gegen eine Beteiligung der Glykolyse bei ausreichender Sauerstoffzufuhr sprechen

1 ein Anteil des MP_2 ist unter Sauerstoff Beatmung auch im Experiment nicht erkennbar Eine Jodacetatinhibierung ist bei 10^{-2} und 10^{-1} mM Konzentration beim Meerschweinchen uberhaupt nicht, ab 5 mM Konzentration wenn uberhaupt, nur sehr schwach nachweisbar Bei dieser Konzentration ist aber schon an eine Beeinflussung des Atmungsstoffwechsels zu denken
2 bei erhohter Lärmexposition ist kein Anstieg des Lactatspiegels in der Perilymphe nachweisbar (Haupt et al , 1974),
3 die LDH Untergruppenverteilung (Abb 2) ist bei vielen Arten rein aerob, bei anderen Arten (Mensch, Ratte) muß nach biochemischen vergleichenden Betrachtungen auf eine generell geringere Leistungsfähigkeit und nicht auf eine gestiegene Glykolysebeteiligung geschlossen werden,



Abb 2 Prozentualer Anteil der LDH H Untereinheit bei verschiedenen Arten

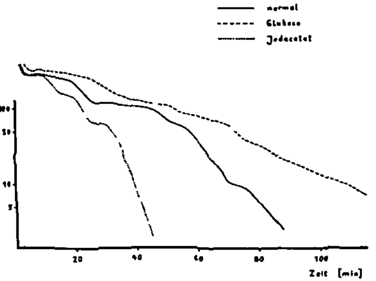


Abb 3 MP_2 Ableitung beim Meerschweinchen

ein Absinken der Glukose – und Glykogen-Reserve beim Meerschweinchen ist erst 10 Min post mortem nachweisbar (Matschinsky & Thalmann, 1967),

einige Arten verfugen uber gar keine Glykogenvorrate im Innenohr, z B Maus, Fledermaus und Katze (ISHII et al , 1969) *Das druckt sich auch, im* MP_2 *Verlauf aus* Die Ratte zeigt ein dem Meerschweinchen ahnliches Bild, der Abfall erfolgt jedoch wesentlich schneller Bei der Maus hingegen ist praktisch überhaupt kein MP_2 zu erkennen

Aus allem ist zu entnehmen, daß die Sauerstoffversorgung auch für das menschliche Innenohr unbedingt lebensnotwendig ist Ein Ausfall könnte von ihm nur für etwa 5–20 Min toleriert werden und auch nur dann, wenn das menschliche Innenohr über Glykogenvorrate verfügt Wir hoffen in Kürze diese Wissenslücke schließen zu können Mit unserer Aussage stimmt auch überein, daß wir durch eine medikamentöse Beeinflussung des Innenohrs wohl schwerhörigen Patienten noch teilweise helfen können, bei völliger Ertaubung aber alle Maßnahmen zu spät kommen

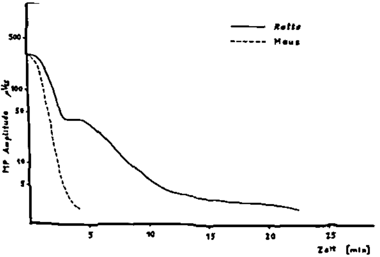


Abb 4 MP_2-Ableitung bei Ratte und Maus

Jedwede therapeutische Unterstützung der Sauerstoffversorgung ist deshalb ein Schritt in dieser richtigen Richtung. Die wirkungsvollste Methode für eine Hilfe bei eingetretenem Schaden und noch mehr als Prophylakticum fur das Cortiorgan zu finden ist unser derzeitiges Hauptanliegen.

SUMMARY

The inner ear function is bound up with a permanent oxygen supply The evidence of it are the patterns of the LDH subunits in inner-ear of eight species The inner ear energy turnover of glycolysis post-mortem was demonstrated at those species possessing glycogen storages The MP_s are alterated by perfusion of glucose or iodoacetic acid During oxygen respiration the MP are not decreased by iodoacetic acid

LITERATUR

Davis, H, Tasaki, I, Smith, C A & Deatherage, B H 1955 Cochlear potentials after intracochlear injections and anoxia *Fed Proc 14*, 35

Deutsch, E 1964 Cumulative effects of oxygen lack on the electrical phenomena of the cochlea *Ann Otol Rhinol Laryngol 73*, 348

Falbe-Hansen, J 1963 Histochemical studies on glycogen in the cochlea of the normal guinea pig *Acta Otolaryngol* (Stockh) *56* 429

Faltynek, L & Vesely, C 1966 The effect of oxygenlack on the microphonic potentials of the guinea pig *Cesk Otolaryngol 15* 260

Haupt, H Scheibe F & Hache, U 1974 Erste Untersuchungsergebnisse uber die Lactatkonzentration in der Perilymphe normaler und larmbelasteter Meerschweinchen Vortrag IV Symposion über Cochleaforschung, Halle (Saale) April 1974

Honrubia, V Johnstone, B M & Butler, R A 1965 Maintenance cochlear potentials during asphyxia *Acta Otolaryngol* (Stockh) *60* 105

Ishii, D, Takahashi T & Balogh, K 1969 Glycogen in the inner ear after acoustic stimulation *Acta Otolaryngol* (Stockh) *67* 573

Konishi, T, Butler, R A & Fernández, C 1961 Effect of anoxia on cochlear potential *J Acoust Soc Am 33* 349

Konishi, T, Kelsey, E & Singleton, G T 1967 Negative potential in scala media during early stage of anoxia *Acta Otolaryngol* (Stockh) *64* 107

Lawrence, M & Arbor, A 1965 Fluid balance in the inner ear *Ann Otol Rhinol Laryngol 74* 486

Lotz, P, Kuhl, K D & Brauer, D 1973 Vergleich der Stoffwechselaktivitäten von Mittel- und Innenohr bei verschiedenen Arten In Jakobi, H & Lotz, P (Herausg), *Funktion und Therapie des Innenohrs* S 155–158 J A Barth-Verlag, Leipzig

Lotz, P, Kuhl, K -D, Brauer, D & Hanson, J 1971 Der Energiestoffwechsel des Innenohres bei verschiedenen Arten *Wiss Z Univ Halle* (Saale), *20 M* 74

Löw, J 1973 Untersuchungen über das postmortale Mikrophonpotential In Jakobi, H & Lotz, P (Herausg), *Funktion und Therapie des Innenohrs*, S 271–274 J A Barth-Verlag, Leipzig

Matschinsky, F M & Thalmann, R 1967 Quantitative histochemistry of microscope structures of the cochlea II Ischemic alterations of levels of glycolytic intermediates and cofactors in the organ of Corti and stria vascularis *Ann Otol Rhinol Laryngol 76*, 638

Schätzle, W 1971. *Histochemie des Innenohres* München, Berlin, Wien Urban und Schwarzenberg

Smith, C A 1958 Structure of the stria vascularis and the spiral ligament. *Ann Otol Rhinol Laryngol 66*, 521

Stack, C R & Webster, D B 1971 Glycogen content in the outer hair cells of kangaroo rat (D spectabilis) cochlea prior and following auditory stimulation *Acta Otolaryngol* (Stockh) *71*, 483

Thalmann, R 1973. Möglichkeiten der quantitativen Mikroanalyse von Cochleastrukturen In Jakobi, H & Lotz, P (Herausg), *Funktion und Therapie des Innenohrs* S 137–154 J A Barth Verlag, Leipzig

Thalmann, R, Bornschein, H & Krejci, F. 1963 Elektrophysiologische Untersuchungen über die Wiederbelebungszeit der Cochlea *Monatsschr Ohrenheilkd 97*, 327

Thalmann, I, Matschinsky, F M & Thalmann, R 1970 Quantitative study of selected enzymes involved in energy metabolism of the cochlear duct *Ann Otol Rhinol Laryngol 79*, 12

Tsunoo, M & Perlmann, H B 1964 Venous obstruction of the cochlea blood flow and function *Acta Otolaryngol* (Stockh) *58*, 127

Vinnikov, J A & Titova, L K 1962 Histochemische Untersuchungen des Cortischen Organs *Z Mikrosk Anat Forsch 69*, 42

Vosteen, K H 1961 Neue Aspekte zur Biologie und Pathologie des Innenohres *Arch Ohren Nasen Kehlkopfheilkd 178* 1

— 1964 Elektronenmikroskopische Untersuchungen über die Verteilung von Glykogen im Ductus cochlearis bei Meerschweinchen *Pract Otorhinolaryngol* (Basel) *26*, 400

Wersäll, J, Hilding D & Lundquist, P-G 1961 Ultrastruktur und Innervation der cochlearen Haarzellen *Arch Ohren Nasen Kehlkopfheilkd 178*, 106

Prof Dr sc med H Jakobi
Leninallee 12
402 Halle/S
DDR

Acta Otolaryngol 82 99–105, 1976

PROTEINSTUDIE ZUR FRAGE DER PERILYMPHSUBSTITUTION DURCH LIQUOR CEREBROSPINALIS VIA AQUAEDUCTUS COCHLEAE

E G Kleinschmidt und U Vick

Aus der Hals Nasen Ohrenklinik der Universitat Rostock Rostock DDR

(Eingegangen am 9 Juni 1975)

Abstrakt Der Gesamtproteingehalt in der Perilymphe der Scala tympani und der Scala vestibuli sowie im Liquor cerebrospinalis wurde unter verschiedenen Bedingungen der Probenentnahme am Meerschweinchen untersucht Aus den gefundenen Proteinkonzentrationen – mit oder ohne subarachnoidaler Punktion vor der Perilymphgewinnung bei geringer und großerer tympanaler Probenentnahme bei fraktionierter Probengewinnung und bei Entnahme post mortem – wird geschlußfolgert daß auch unter physiologischen Bedingungen durch den Liquornachfluß über den Aquaeductus cochleae eine Verdunnung der Proteinkonzentration der tympanalen Perilymphe zumindest in der Basalwindung erfolgt Die Proteinkonzentration des Liquors ist etwa vierfach geringer bezogen auf den Durchschnittsgehalt der Perilymphe und eignet sich deshalb gut fur Studien dieser Art In der vestibularen Perilymphe wurde gegenuber dem Gesamtinhalt der Scala tympani eine signifikant hohere Proteinkonzentration gefunden

Neben der Ansicht, die Perilymphe sei hauptsachlich ein Ultrafiltrat des Blutes (Schindler, 1965, Schnieder, 1975), vertreten viele Autoren die sich gerade in jungerer Zeit mit der Untersuchung dieser Problematik beschaftigten die Meinung die Perilymphe rekrutiere sich vornehmlich aus dem Liquor cerebrospinalis Als Zuflußwege werden die Perivaskular und Perineuralraume (VIII Hirnnerv) hauptsachlich aber der Aquaeductus cochleae angesehen (Arnold & v Ilberg 1971, Arnold 1974, Federspil & Gloebel 1974 Wicke & Firbas, 1974) Zur Klarung dieser Verhaltnisse wurden bisher folgende Methoden herangezogen Biochemische Vergleichsuntersuchungen zwischen Liquor und Perilymphe (Palva & Raunio, 1971, Makimoto & Silverstein 1974), Registrierung der Druckverhaltnisse und des Druckausgleichs zwischen den Raumen des Liquor und Perilymphsystems (Gestewitz, 1971, Beentjes, 1972), Verfolgung der Ausbreitung von Tracersubstanzen, die in den Subarachnoidalraum, in die Innenohrflüssigkeiten oder in das Blutgefaßsystem eingebracht werden (Sando et al , 1971 Komaki, 1972, Juhn & Guzowski, 1973, Silverstein et al , 1973), Erforschung des strukturellen Aufbaus des Aquaeductus cochleae und die Auswirkungen eines experimentellen Verschlusses (Anson et al , 1965, Palva & Dammert, 1969, Kimura et al , 1974) Fur eine endgultige Klarung ist eine extreme Verfeinerung der analytischen Ultramikromethoden und eine Beherrschung der Probenentnahmetechnik notwendig Zum anderen setzt aber die exakte Beherrschung der Entnahmetechnik eine genaue Kenntnis der Zirkulationsverhaltnisse der Innenohrflussigkeiten voraus Stromung, Resorption und Austausch mit anderen Flussigkeitssystemen lassen regionale Konzentrationsunterschiede entstehen Damit gewinnt der Ort der Probenentnahme an der Cochlea erhebliche Bedeutung

Es wurde versucht, uber die Bestimmung des Gesamtproteingehalts im Liquor und in

der Perilymphe der Scala tympani (fraktionierte Entnahme) sowie der Scala vestibuli einen Beitrag zur Klärung des Problems zu liefern Die Proteinbestimmung bietet sich hierfür an da nach den bisherigen Erfahrungen die Konzentration in der Perilymphe etwa vierfach höher ist als im Liquor Als Versuchstiere dienten Meerschweinchen Die Proteinbestimmung erfolgte in Anlehnung an die Methode von Lowry et al (1951) fotometrisch in Mikrokuvetten Für eine Bestimmung wurde 1 μl Perilymphe oder Liquor benötigt

METHODE

Meerschweinchen (Gewicht zwischen 200 und 250 g positiver Ohrmuschelreflex) wurden in Äthylurethan Narkose präpariert

Serie I Nach Eröffnung der Bulla erfolgte ohne weitere Vorpräparation die Perilymphgewinnung in der weiter unten beschriebenen Weise

Serie II Vor Eröffnung der Innenohrräume wurde subokzipital punktiert Dazu wurde die Membrana atlanto-occipitalis dargestellt und mit einer spitz ausgezogenen Glaskapillare vorsichtig durchstoßen Nach Abfließen des unter Druck stehenden Liquors (gleichzeitig Probengewinnung für die Proteinbestimmung) wurde mit spitz ausgezogenen Kapillaren unter mikroskopischer Sicht und mittels Mikrokolbenburette Perilymphe aus der Cochlea gewonnen Sofort nach Eröffnung der Cochlea am Helicotrema wurde tympanale Perilymphe durch das runde Fenster entnommen (entweder in zwei Fraktionen möglichst schnell hintereinander oder in unterschiedlicher Menge zwischen 1 und 2 5 μl bzw zwischen 3 und 5 μl) Anschließend erfolgte durch ein Bohrloch in der Basalwindung der Scala vestibuli die Entnahme vestibulärer Perilymphe

Serie III Dekapitation der Tiere Herausbrechen des Felsenbeines mit Freilegung der Cochlea Eröffnung am Helicotrema fraktionierte tympanale Perilymphentnahme durch das runde Fenster und anschließend vestibuläre Perilymphgewinnung durch ein Bohrloch in der Basalwindung Sämtliche Operationen wurden innerhalb 10 min nach Dekapitation ausgeführt

Nach beidseitigem Zuschmelzen der Kapillaren erfolgte Bestimmung der Blutbeimengung über die Ermittlung der Erythrozytenzahl Dazu wurden die Proben in den Abnahmekapillaren (gleicher Innendurchmesser[1]) nachdem sich die Erythrozyten bei waagerechter Lagerung der Kapillaren abgesetzt hatten unter dem Mikroskop beurteilt und mit einer Standard Verdünnungsreihe bekannten Erythrozytengehalts unter gleichen Bedingungen verglichen Stufenweise Zuordnung der Perilymph und Liquorproben bezüglich Blutbeimengung nach einer Prozentskala von 0 2 0 1 0 05 0 0? 0 01 0 005 und 0 001 wurde vorgenommen Proben mit einem Blutgehalt über 0 1% wurden nicht verwertet Nach Abschätzen des Blutgehalts wurden die Kapillaren zentrifugiert (2 min bei 15 000 U/min) und durch Abschneiden der Kapillarspitze vom Erythrozytensediment entfernt Mit den auf 1 μl geeichten Microcaps erfolgte die Dosierung der Proben Unter Beachtung der von Glasser & Kleine (1962) erarbeiteten Verbesserungen erfolgte 30 min nach Phenolreagenz Zugabe die kolorimetrische Messung in 1 cm Mikrokuvetten bei 750 nm gegen einen Leerwert (statt 1 μl Probenflüssigkeit 1 μl Aqua b dest) Bei jeder Bestimmungsreihe wurden zur Überprüfung der Eichkurve (Human Albumin) 3 Testansätze entsprechend 25 100 und 500 mg/100 ml mitbestimmt

ERGEBNISSE UND DISKUSSION

Eine Kommunikation zwischen Subarachnoidalräumen und Perilymphräumen kann auf Grund der vielfachen experimentellen Befunde als gesichert angesehen werden Dem Aquae-

[1] 5 μl Wiretrol Micropipettes 1 μl Microcaps Drummond Scientific Company USA

abelle I *Proteingehalt der Perilymphe bei fraktionierter tympanaler Entnahme Liquorraum nicht eroffnet*

ty I			ty II			ve		
µl	%	c	µl	%	c	µl	%	c
1,5	0 005	46	3	0 1	137	1,5	0 01	196
1 5	0 01	51	3	0 01	85	1 5	0 01	238
1 5	0 005	33	1 5	0 005	50	2	0 01	200
1 5	0 001	45	2	0 02	101	1,5	0 005	227
1 5	0 01	93	2	0 1	121	1,5	0 01	232
2	0 005	40	2	0 005	55	2	0 05	240
1 5	0 005	33	4 5	0 01	34	2	0 01	150
		49			96			?12
		20 7			41 0			32 5

y I erste Fraktion der tympanalen Perilymphe Abnahme durch das runde Fenster ty II zweite Fraktion ve vestibulare erilymphe µl Probenmenge in Mikroliter % Blutbeimengung in % c Proteingehalt der Proben in mg/100 ml m ittelwert s Standardabweichung

uctus cochleae muß eine entscheidende Rolle ei der Verbindung beider Systeme zugemes en werden wie auch die Untersuchungen von omaki (1972) und Moscovich et al (1973) er aben Die Durchlassigkeit wurde hauptsach ich an Katzen und Meerschweinchen unter ucht und bestatigt Arnold (1974) betont aß sich im Gegensatz zu fruheren Ansichten us neueren Untersuchungen auch fur den Menschen eine entsprechende Bedeutung des Aquaeductus cochleae fur die Perilymph substitution durch Liquor ergibt Die Aus tauschvorgange scheinen von artspezifischen und individuellen Eigenschaften des Aquae ductus cochleae abhangig zu sein (Wicke & Firbas 1974)

Tonndorf (1972) spricht von einem 3 Fenster' des Innenohres und meint damit die Kommunikationsmoglichkeit uber den Aquae ductus sowie die perineuralen und perivas kularen Kanale Nach Schaltenbrand (1949) und Arnold (1974) sollten die Perilymphraume als Extrazellularraume verstanden werden welche in die offene Verbindung zwischen extrazellularer Flüssigkeit Liquor' und Extrazellularflussigkeit Lymphe einge schaltet sind An verschiedenen Stellen finden Vorgange der Resorption und der an reichernden Produktion statt Auch über die Abflußmoglichkeiten zum Lymphsystem wurden in den letzten Jahren Vorstellungen erarbeitet (Musebeck, 1965, Arnold & v Il berg 1971, 1972, Arnold 1971, 1974)

Aus dem bisher Dargelegten ergibt sich die Notwendigkeit des Auftretens biochemischer Konzentrationsunterschiede Eine Bestatigung in dieser Richtung erbrachten kurzlich Maki moto & Silverstein (1974) mit dem Nachweis daß der Elektrolytgehalt von tympanaler und vestibularer Perilymphe erheblich differiert Sie fanden, daß bei Katzen die Konzentration an Natrium und Kaliumionen der Perilymphe aus der basalen Windung der Scala tympani dem Liquor ahnlicher ist als der vestibularen Perilymphe

Unsere Versuche ergaben daß der Protein gehalt der tympanalen Perilymphe signifikant niedriger ist im Vergleich zur vestibularen Perilymphe Die bei Rauch (1964) zusammen gestellten Angaben zur Meerschweinchen perilymphe und weitere Literaturangaben be ziehen sich auf eine summarische Proteinkon zentration und reichen von 42 mg/100 ml bis zu 400 mg/100 ml (Schindler et al 1965 Schnieder, 1968) Rauch differenziert die Peri lymphe des Meerschweinchens und gibt 58 mg/100 ml fur die Scala tympani und 114 mg/ 100 ml fur die Scala vestibuli an Scheibe et al (1975) finden im Proteingehalt keinen signifikanten Unterschied zwischen beiden Scalen, sie diskutieren Werte zwischen 100 und 200 mg/100 ml

Tabelle II *Proteingehalt des Liquor cerebrospinalis beim Meerschweinchen*

	1	2	3	4	5	6	7	8	9
%	0 01	0 01	0 01	0 001	0 005	0 01	0 001	0 01	0 01
c	32	38	28	43	53	30	37	38	42

m=38 mg/100 ml s=7 2 Bedeutung der Symbole siehe Tabelle I

Bei Katzen fand Silverstein (1966) unterschiedliche Proteinkonzentrationen in den Scalen, namlich tympanal 160 und vestibular 273 mg/100 ml Auch Palva & Raunio (1969) fanden beim Menschen, allerdings 1 bis 2 Tage post mortem mit Werten von 280 mg/ml fur die Scala tympani und 312 mg/100 ml fur die Scala vestibuli eine betrachtliche Differenz

Bei zweimaliger Entnahme zeitlich unmittelbar hintereinander aus der Scala tympani durch die runde Fenstermembran (Tabelle I) wurde in den ersten Proben eine mit durchschnittlich 49 mg/100 ml sehr geringe Proteinkonzentration gefunden, die Beziehungen zum Liquor cerebrospinalis vermuten laßt

Fur den Liquor ergab sich eine durchschnittliche Proteinkonzentration von 38 mg/100 ml (Tabelle II) Citron et al (1956) geben 20 mg/100 ml Schindler et al (1965) 210 mg/100 ml an Fur menschlichen Liquor cerebrospinalis nennt Rauch (1964) 20–30 mg/100 ml nach Ruedi et al (1965) besteht eine Konzentration von 18 mg/100 ml Der Subarachnoidalraum wurde von uns bei den Tierversuchen der Serie I nicht eroffnet, so daß es wahrscheinlich ist, daß bei Eroffnung des Perilymphraumes Liquor durch den Aquaeductus cochleae nachfloß und sich in seinem kochlearen Mundungsbereich mit Perilymphe der Basalwindung vermischte

Der Proteinmittelwert der zweiten Proben die schnell durch Abziehen der restlichen tympanalen Perilymphe gewonnen wurden betragt 96 mg/100 ml Hierbei durfte es sich um Perilymphe aus den oberen Schneckenwindungen handeln (am Helicotrema war eroffnet worden), allerdings auch vermischt *mit nachfließendem Liquor* Zwischen den

Tabelle III *Proteingehalt der Perilymphe bei unterschiedlicher tympanaler Entnahmemenge Liquorraum vorher eroffnet Blutbeimengung aller Proben kleiner als 0 1 %*

Serie A

	Tympanal		Vestibular	
	μl	c	μl	c
1	2 5	77	2 5	221
2	2 5	97	3	184
3	2	193	2	182
4	1 5	77	1 5	243
5	2	72	2	165
6	2	58	2	152
7	2,5	80	3 5	205
8	2	100	5 5	220
9	2 2	101	3	273
10	2	143	3	170
m		100		202
s		40 2		43 0

Serie B

	Tympanal		Vestibular	
	μl	c	μl	c
1	4 5	103	5 5	103
2	4 5	108	3	243
3	5	213	2 5	240
4	3	151	3	200
5	4	145	3	212
6	3	112	3	206
7	4	140	4	212
8	3	169	2	287
9	5	213	4 5	190
10	3 5	80	3 5	232
11	3 5	117	3 5	205
12	4	147	3	133
m		142		205
s		41,5		48 7

Serie A tympanale Probe 1–2 5 μl vestibulare Probe durchschnittlich 2 8 μl Serie B tympanale Probe 3–5 μl vestibulare Probe durchschnittlich 3 6 μ Bedeutung der Symbole siehe Tabelle I

Tabelle IV *Postmortaler Proteingehalt der Perilymphe bei fraktionierter tympanaler Entnahme 10 min post mortem*

	ty I			ty II			ve		
	µl	%	c	µl	%	c	µl	%	c
1	1 5	0 02	109	2 5	0 005	146	1 5	0 0?	145
2	1,5	0 02	125	–	–	–	1 5	0 01	253
3	1,5	0 005	173	2	0 02	211	1,5	0 05	269
4	1 5	0 01	102	1 5	0 1	149	1 5	0 01	214
5	2	0 01	143	1 5	0 01	165	–	–	–
6	1 5	0 05	156	2 5	0 01	151	1,5	0 005	231
7	2	0 1	170	2 5	0 02	256	1 5	0 01	213
8	1,5	0 001	199	2,5	0 001	217	1,5	0 002	299
9	2	0 1	133		–	–	–	–	–
10	1 5	0 1	72	–	–	–	–	–	–
1	1 5	0 005	140	2 5	0 005	246	1 5	0 1	225
2	1,5	0 002	237	2 5	0 1	221	1,5	0 005	229
m			147			196			231
S			47 4			43 4			42 7

Bedeutung der Symbole siehe Tabelle I

beiden Durchschnittswerten aus dem basalen und oberen Bereich der Scala tympani 49 und 96 mg/100 ml (Tabelle I) besteht nach dem *t* Test ein signifikanter Unterschied mit 95% Wahrscheinlichkeit Zwischen beiden Werten besteht zur Proteinkonzentration der Scala vestibuli mit durchschnittlich 212 mg/100 ml ein signifikanter Unterschied von uber 99% Wahrscheinlichkeit

Zur Ausschaltung oder wenigstens Herab setzung des Liquornachflusses wurde vor Er offnung der Innenohrraume der Liquorraum punktiert Theoretisch mußte durch den abfließenden Liquor eine Druckentlastung des Aquaeductus erreicht werden Die hoheren tympanalen Proteinwerte (Tabelle III) schei nen dies zu bestatigen Bei Entnahme kleinerer Proben wurde im Durchschnitt 100 mg/100 ml gefunden bei großerer Probenmenge d h bei Miterfassen der tympanalen Perilymphe der oberen Windungen 142 mg/100 ml In beiden Fallen (Serie A und Serie B in Tabelle III) er gibt sich nach dem *t* Test zwischen den Mittel werten der tympanalen und vestibularen Proteinkonzentration ein signifikanter Unter schied mit uber 99% Wahrscheinlichkeit Auch unter physiologischen Bedingungen scheint im basalen tympanalen Schneckenteil eine ge ringere Eiweißkonzentration vorzuliegen als in den aufsteigenden Schneckenwindungen

Um vollig sicher zu sein daß wahrend der Perilymphabnahme keine Beeinflussung durch Liquor uber den Aquaeductus mehr moglich ist, wurden bei einer weiteren Serie die Tiere getotet und die Felsenbeine herausgelost Nach Untersuchungen von Silverstein & Griffin (1970) an Katzen ist die Veranderung der Proteinkonzentration in der Perilymphe post mortem sehr gering Die nach Aufbrechen der Bulla in gleicher Weise wie bei den anderen Serien und in einem Zeitraum von 4–9 min post mortem gewonnenen Perilymphen ergaben noch hohere Proteinwerte (Tabelle IV) Interessant ist aber, daß auch hier bei der fraktionierten tympanalen Perilymphgewin nung ein Gradient in der Proteinkonzentration gefunden wurde im Sinne einer Proteinzu nahme zur Schneckenspitze Die statistische Auswertung nach dem *t* Test ergab fur die Mittelwerte der ersten und zweiten Fraktion aus der tympanalen Scala, 147 und 196 mg/100 ml eine signifikante Differenz mit 95% Sicher heit Faßt man die postmortalen Werte fur die Proteinkonzentration aus erster und zweiter tympanaler Fraktion zusammen, so im Durchschnitt fur die Scala ty

mg/100 ml Dieser Wert ist nach dem *t*-Test mit einer statistischen Wahrscheinlichkeit von etwa 97% verschieden vom vestibularen Mittelwert fur die Proteinkonzentration 231 mg/100 ml Es ist anzunehmen, daß ent sprechende Verhaltnisse auch unter physio logischen Bedingungen vorliegen

Mit Ausnahme einer sich andeutenden Ten denz der Zunahme post mortem, wurde bei allen Untersuchungen fur die Perilymphe der Scala vestibuli praktisch der gleiche Wert ge funden Aus unseren Versuchen erhellt die Problematik der Perilymphgewinnung spe ziell aus der Scala tympani Eine besonders kritische Betrachtungsweise erfordert die Beurteilung der Perilymphe der Basalwin dung

Bei allen Untersuchungen muß die Existenz des Aquaeductus cochleae und der durch ihn in das Perilymphsystem einfließende Liquor cerebrospinalis berucksichtigt werden

SUMMARY

Total protein contents in the perilymph of Scala vestibuli and Scala tympani as well as in the cerebrosp nal fluid (CSF) of guinea pigs were determined by which speci mens were taken under the following various conditions With or without subarachnoidal puncture before peri lymph collection by varying the amount of perilymph taken from Scala tympani by fractionating collection and under post mortem cond t on The results suggest that under physiological cond tions the CSF also flows through the cochleae aqueduct and the protein concentra tion in the Scala tympani decreases especially in the basal winding *Because the protein content in CSF is in average* four times lower than in fluids of the inner ear it is of pref erence to consider the quest on of connection between the CSF and the fluids of the inner ear In the specimens taken the blood contam nation was estimated on the basis of erythrocytes in the connect on cap llaries under micro-scope The protein content in the perilymph of Scala ves tibuli was found significantly higher in relat on to peri lymph of Scala tympani

LITERATUR

Anson B J Donaldson J A Warpeha R L & Winch T R 1965 The vestibular and cochlear aque ducts Their variational anatomy in the adult human ear *Laryngoscope 75* 1203

Arnold W 1971 Die Bedeutung des subep thelialen Rau mes der Mittelohrschleimhaut *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 198* 262

— 1971 [illegible]

Arn [illegible] zwischen Liquor und Perilymphraum *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 198* 247

— 1972 Neue Aspekte zur Morphologie und Funktion des runden Fensters *Z Laryngol Rhinol Otol 51* 390

Beentjes B I J 1972 The cochlear aqueduct and the pressure of cerebrospinal and endolabyrinthine fluids *Acta Otolaryngol* (Stockh) *73* 112

Citron L Exley D & Hallpike C S 1956 Zit nach Rauch S Federspil P & Gloebel B *1974 Uber* die Zusammenhange zwischen Liquor cerebro-spinalis und Innenohrflussigkeiten *Monatsschr Ohrenheilkd 108* 526

Gestewitz H R 1971 Pulsierende Bewegungen am runden und ovalen Fenster als Ausdruck von Peri- und Endolymphbewegungen *Wiss Z Univ Halle 20* 135

Glasser D & Kle ne R 1962 Beitrag zur Eiweißbe-stimmung in stark verdunnten Losungen *Pharmazie 17* 32

Juhn S K & Guzowski J 1973 The passage of intra-cisternally injected albumin and urea into perilymph *Acta Otolaryngol* (Stockh) 76 313

Kimura R S Schuknecht H F & Ota C Y 1974 Blockage of the cochleae aqueduct *Acta Otolaryngol* (Stockh) 77 1

Komaki Y 1972 Glucose Transport in the inner ear *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 201* 258

Lowry O H Rosebrough N J Farr A L & Ran dall R J 1951 Protein measurement w th the Fol n Phenol Reagent *J Biol Chem 193* 265

Makimoto K & S lverstein H 1974 Sod um and potas sium concentrations in the endolymph and perilymph of the cat *Ann Otol Rhinol Laryngol 83* 174

Moscovitch D H Gannon R P & Laszlo M 1973 Perilymph displacement by cerebrospinal flu d in the cochlea *Ann Otol Rhinol Laryngol 82* 53

Musebeck K 1965 Die perivascularen Lymphspalten des Innenohres *Arch Ohren Nasen Kehlkopfheilkd 184* 560

Palva T & Dammert K 1969 Human cochlear aque-duct *Acta Otolaryngol* (Stockh) Suppl *246*

Palva T & Raunio V 1969 The total prote n of human perilymph *Acta Otolaryngol* (Stockh) 67 211

— 1971 CSF specific proteins in perilymph *Acta Oto-laryngol* (Stockh) 72 421

Rauch S 1964 *B ochem e des Hororgans* Verlag G Thieme Stuttgart

Ruedi L Sanz M C & Fisch U 1965 Untersuchung der Perilymphe nach Stapedektom e in Otosklerose-fallen *Acta Otolaryngol* (Stockh) 59 289

Sando I Wood R P Masuda Y & Hemenway W G 1971 Perilymphat c commun cation routes in gu nea pig chochlea *Ann Otol Rhinol Laryngol 80* 826

Schaltenbrand G 1949 Anatomie und Physiolog e der Liquorzirkulation *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 156* 1

Scheibe F Haupt H Hache U Gerhard H J &

Lauterbach, H 1975 Zur Proteinkonzentration der Meerschweinchen Perilymphe *Acta Otolaryngol* (Stockh) *79*, 51

hindler, K 1965 Perilymphe als Ultrafiltrat des Serums *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 185*, 586

hindler, K, Schmieder, E A & Wullstein, H L 1965 Vergleichende Bestimmung einiger Elektrolyte und organischer Substanzen in der Perilymphe otosklerosekranker Patienten *Acta Otolaryngol* (Stockh) 59 309

hmieder, E-A 1968 Morphologische und biochemische Befunde nach Stapedektomie beim Meerschweinchen *Arch Klin Exp Ohren Nasen Kehl kopfheilkd 191*, 709

1975 A contribution to the physiology of the perilymph Part I The origin of perilymph *Ann Otol Rhinol Laryngol 76* 76

lverstein, H 1966 Biochemical studies in the inner ear fluids in cats *Ann Otol Rhinol Laryngol 75* 48

Silverstein, H & Griffin, W. L. jr 1970 Comparison of inner ear fluids in the antemortem and postmortem state of the cat *Ann Otol Rhinol Laryngol 79*, 178

Silverstein, M D, Naufal, P & Belal, A 1973 Causes of elevated perilymph protein concentrations *Laryngoscope 83*, 476

Tonndorf, J 1972 Zitiert nach Wicke, W & Firbas, W

Wicke, W. & Firbas, W 1974 Experimentelle Untersuchungen der perilymphatischen Verbindungswege bei der Katze *Arch Otorhinolaryngol 208*, 267

Dr E -Günther Kleinschmidt
HNO-Klinik der Universität Rostock
Doberaner Straße 137/139
25 Rostock
DDR

Acta Otolaryngol 82 106–111, 1976

CEREBELLOPONTINE ANGLE TUMOURS, OTHER THAN ACOUSTIC NEUROMAS

A Report on 34 Cases
A Presentation of 7 Bilateral Acoustic Neuromas

J Thomsen

From the University ENT and Neurosurgical Departments Rigshospitalet Copenhagen Denmark

(Received August 15 1975)

Abstract The symptoms preoperative diagnostic results and histological findings in 34 cerebellopontine angle tumour cases other than acoustic neuromas are presented The meningeomas dominate by far this group of tumours The symptomatology is much less uniform than that of the acoustic neuromas where the VIII nerve is dominant The facial nerve is less involved whereas the other cranial nerves give symptoms more frequently One remarkable sign is the presence of severe trigeminal neuralgia which we have not encountered in acoustic neuromas Only 35% of the non neuroma patients had elevated spinal fluid protein compared with 100% in our acoustic neuroma cases Furthermore 7 patients with bilateral acoustic neuromas are presented The connection with von Recklinghausens disease and multiple meningeomas is discussed

In previous papers we have reported the diagnostic and surgical findings in 125 patients with acoustic neuromas operated on during the period 1957–1972 at the neurosurgical department Rigshospitalet Copenhagen

During the same period 34 tumours other than neuromas have been encountered in the cerebellopontine angle In this paper we report the diagnostic findings in these tumour cases with special reference to the differences between the acoustic neuromas and these 'other' tumours The tumour pathologies are listed and discussed

This paper has been supported by a grant from the P Carl Petersen Foundation (Grant no B 9[illegible])

In addition, this report deals with 7 patients with bilateral acoustic neuromas

PATIENTS AND RESULTS

Of the 34 patients in the present series, 4 were younger than 30 years and 10 older than 60 A 2 year old baby was the youngest, a man of 73 the oldest patient The majority were in the age group 30–60 years There are twice as many women as men (Fig 1) 30% were seen within the first year of the disease and 55% between one and 5 years after the debut of the symptoms "Only" 15% had had symptoms longer than 5 years (Table I) In Table II the patients' indications of their first

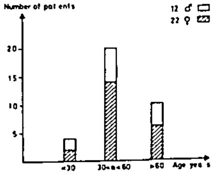


Fig 1 Age distribution of 34 patients with cerebellopontine angle tumours other than acoustic neuromas

able I *Length of history in 34 patients with rs of the cerebellopontine angle (non uromas)*

year	1 5 years	5 years
0	19	5
J c)	(55%)	(15%)

ymptoms are set out It should be noted that earing loss and vertigo only accounts for just ss than 50% 20% started by suffering from eadaches while 5 patients (15%) reported neuralgia The cranial nerve findings t the time of diagnosis are shown in Fig 2 7% had normal hearing 50% normal caloric eaction The remainder of the patients had ing degrees of hearing loss and canal pare is 12% showed spontaneous nystagmus ₀% positional nystagmus

Facial and trigeminal nerve involvement the diagnosis are shown in Fig 3 The sen ory function of the facial nerve is evaluated , testing the intermedius nerve 26% had deficiency on the anterior two-thirds of tongue while 38% had diminution of the olacrimal reflex (Thomsen & Zilstorff, ɔ) Four patients (12%) had facial palsy 44% had diminished corneal sensibility ›oberg Ans 1955) and 29% diminished fa sensitivity Two patients only (6%) had geminal motor deficiency

In 8 patients (24%) there was f cranial nerve involvement II or VIII in order of II IV, III, I

Fig 4 indicates the fi

ıable II *First subjective ients with tumours of the ıngle (non neuromas)*

Hearing loss
ert go
Headache
Trigeminc a

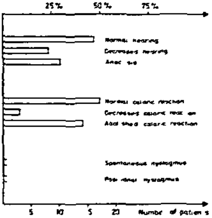


Fig 2 VIII nerve symptoms in 34 patients with cerebellopontine angle tumours other than acoustic neuromas

other cranial nerves spinal fluid protein content and other neurological deficits 59% showed cerebellar symptoms 29% brain stem symptoms 15% were mentally deteriorated 24% had increased intracranial pressure evaluated by choked discs while 35% had elevation of the spinal fluid protein content

Table III sets out the distribution of histological diagnoses in 33 of the 34 patients The last patient only had a radiologically verified tumour eroding the petrous apex No histological diagnosis was available It is clearly that the meningeomas by far dominate of tumours 12% of the total num- ellopontine angle tumours en our department (125 acoustic › 34 other tumours) They are listributed between the endo- fibromatous and transitional ous meningeomas were

h meningeomas had in association One gone removal of an ast 5 years prior to) His main symp- il neuralgia head s At operation a ɔma was removed , of adeno-

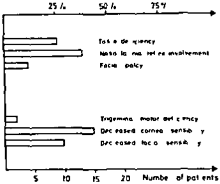


Fig 3 V + VII nerve symptoms in 34 patients with cerebellopontine angle tumours other than acoustic neuromas

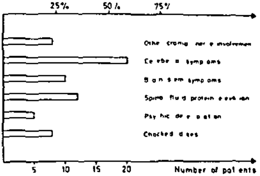


Fig 4 Other neurological deficits in 34 patients with cerebellopontine angle tumours other than acoustic neuromas

carcinoma type Six months later he had signs of recurrence of the angle tumour, and died one month subsequently, without reoperation The other patient was a 54-year-old female who, in addition to a fibromatous meningeoma, also had a meningeal sarcoma and a gliosarcoma of the left temporal hemisphere The patient died one month after the operation (exploratory)

Next in order of frequency are the epidermoids or primary cholesteatomas, and the arachnoid cysts, each group containing 4 patients, 2 5% of the total cerebellopontine angle tumours The youngest patient in the material was a 2-year-old girl, with a malignant anaplastic tumour of mesenchymal type, probably a reticulosarcoma Six days after an attack of acute otitis she developed paralysis of V, VI VII VIII, X, XII cranial nerves X-rays revealed extensive destruction of the cranial base An exploration of the angle revealed the above-mentioned tumour

Another child was a 9 year-old boy Histology showed an ependyblastoma of the 4th ventricle, extending into the cerebellopon tine angle After postoperative radiation the patient lived 2 years until signs of recurrence appeared

A 40 year-old female had a metastatic anaplastic carcinoma However postoperative diagnostic work-up was unsuccessful and the primary tumour was never found

Eight patients were operated upon for recurrence of a tumour (24%) 6 meningeomas one epidermoid and one malignant tumour (adenocarcinoma) Five patients died in connection with the operation or during the post operative period Two were endotheliomatous meningeomas, large and severely bleeding tumours, one patient with a chordoma and 2 patients with malignant tumours Three other patients died, 2 of meningeomas after 6 months and 2 years respectively, due to recurrence of tumour, and one patient with adenocarcinoma metastasis after 6 months also due to recurrence Of the living patients 5 (19%) got a facial palsy

In our report on 125 acoustic neuromas we mentioned 7 patients with bilateral acoustic neuromas Due to the connection between

Table III Histological diagnosis in 34 tumours of the cerebellopontine angle (non neuromas)

Meningeomas		
Endotheliomatous	8	
Fibromatous	7	
Transitional	4	19
Epidermoid (congenital cholesteatoma)		4
Arachnoid cysts		4
Chordoma		1
Reticulosarcoma		1
Ependymoblastoma		1
Ependymoma (cystic)		1
Angio-reticuloma (Lindau s tumour)		1
Anaplastic carcinoma		1
Radiologically verified tumour eroding the petrous apex		
No histology available		1
		34



g 5 Und agnosed acoust c neuroma arrow in the basal rn of the spiral canal a few mm from the round window

ilateral neuromas and meningeomas a re iew of these patients is given here Four of hese patients are fairly young ages 13 14 17 nd 26 years All 4 had signs of von Rech nghausens disease as all had extracranial ieuro-fibromas as well as cafe au lait spots Three patients had signs of other intracranial euromas especially trigeminal neuromas In hese patients there was no family history of ieuro fibromatous disease However the last atient a 27 year old female had a strong fam ly history as when she was 1 year old her nother died of bilateral neuromas as well as nultiple meningeomas This patient further nore as her mother had multiple meningeo nas of the en plaque type She underwent a subtotal removal of a left acoustic neuroma vhen 17 years old resulting in abolished iearing in this ear Gradually she developed ght side hearing loss and a right side acoustic euroma was diagnosed However she was pregnant and at term when admitted to the neurosurgical department She was somno lent with increased intracranial pressure as well as in labour She was transferred to the obstetrical unit where a normal baby was delivered by cesarian section Thereafter the operation for the neuromas was undertaken She died during operation due to intractable bleeding from a very large neuroma and large meningeomas

In this group of patients the number of fe males is prominent as with the unilateral neuromas

The 2 of the remaining 3 patients with bi lateral neuromas had clinical signs of such tu mours They were both operated on both sides and died subsequently to the second operation

The last patient also died after operation but clinically only the left side neuroma was diagnosed before death The other neuroma was found accidentally when examining the temporal bone of the contralateral side A small acoustic neuroma was found in the basal turn of the spiral canal a few mm from the round window (Fig 5) (Thomsen & Jørgen sen 1973)

None of these last 3 patients had extra cranial signs of von Recklinghausens disease

DISCUSSION

The distribution of the non neuromas of the cerebellopontine angle in this material does show the same pattern as in other ma terials (Hitselberger & Gardner 1968 Nager 1967) even though the frequency of meningeo mas is somewhat higher 12% of the total number of angle tumours including the neu romas The term meningeoma was intro duced by Cushing for a group of hamartomat ous not truly neoplastic tumours of the me ninges found to be attached to any of the three meninges It is now generally held that they represent neoplasia of the arachnoid lining cells Arachnoid villi are greatly co centrated in the walls of the major v

sinuses particularly abundant in the sagittal sinus In the cerebellopontine angle the meningeomas are felt to arise from the arachnoid villi in the large and small venous sinuses surrounding the temporal bone (Hitselberger & Gardner 1968) About 8–9% of the intracranial meningeomas are located in the posterior fossa and about 42% of these originate from the posterior surface of the petrous bone (Castellano & Ruggiero 1953) The following main types can be distinguished (Zulch 1957) (1) endotheliomatous (2) fibromatous (3) transitional (4) angiomatous The distribution of our meningeomas is fairly even among the first 3 types while the angiomatous type is not represented A fifth group of meningeomas can be added showing malignant or sarcomatous dedifferentiation Indeed one of our fibromatous meningeomas did show sarcomatous changes

In this report we have in addition to the non neuromas described 7 patients with bilateral acoustic neuromas Four of these very young patients had both signs of other intracranial neuromas as well as signs of peripheral involvement indicating a full blown von Recklinghausens disease This disease was first described in 1882 by von Rechlinghausen being of the opinion that the fibromas of the skin and nerves arose from the peri and endoneurium of mesodermal origin To day it is generally believed that the tumours are of neuro ectodermal origin even though the debate over the nature of the tumour continues Von Recklinghausens disease has frequently been associated with meningeomas often multiple (Nager 1964 Edvard & Passy 1972) In our material we have one patient who had this combination of tumours and also in the family history as her mother died suffering from both neuromas and meningeomas This in accordance with the disease being a Mendelian dominant (Gardner & Turner 1940 Glass & Abbott 1953)

In 3 patients with bilateral acoustic neuromas there was no evidence of other intracranial disease or extracranial involvement It is debated whether this is really von Recklinghausens disease it could be a central form (Nager 1964) However there is no difference in the histological picture of the bilateral acoustic neuromas compared with the unilateral Therefore the unilateral could also be part of the von Recklinghausens disease as an even more isolated central form and the unilateral might more frequently be associated with contralateral asymptomatic neuromas and therefore bilateral as pointed out in an earlier publication (Thomsen & Jorgensen 1973)

The epidermoids or primary cholesteatomas are next in order of frequency which is in agreement with other materials They arise from congenital epithelial inclusion remnants In one case it was possible to suspect such a diagnosis because of the distinctive scalloped contour of the surface shown at Pantopaque cisternography as also pointed out by Hitselberger & Gardner (1968)

When making a comparison of the preoperative examinations and history of the disease between the acoustic neuromas and these non neuromas there are several differences to be pointed out even though it is impossible to be sure of the exact diagnosis before surgery

Age and sex distribution do not differ in any way With regard to the patient s indication of first subjective symptom there is a striking difference 95% of the patients with neuromas had hearing loss and tinnitus as first symptom in contrast to 24% among the non neuromas 4% of the neuromas started with vertigo 24% in the other group In the non neuromas headaches (21%) trigeminal neuralgia (15%) and mental changes (12%) were outstanding symptoms in the debut of the disease while these symptoms did not show up until very late in the history of the neuromas Furthermore the non neuromas" had a definitely shorter disease history

Regarding preoperative examinations it was striking that 50% of the non neuromas had normal hearing and normal vestibular func

while none of the neuromas had normal ng and only 4% normal caloric re n The nervus intermedius function was impaired in this group of tumours, pre d here, as well as trigeminal symp

The other cranial nerves were more ently involved in this group of "non omas" (24%), than were the neuromas) This also applies to the brain stem ptoms, 29% in this group, 10% in the omas There was no difference in the in nce of papilledema

reviewing the neurological findings the wing conclusion can be drawn The ptomatology is much less uniform than of the acoustic neuromas, where the nerve is dominant The facial nerve is involved, whereas the other cranial es give symptoms more frequently A arkable sign is the presence of severe tri inal neuralgia, which we have not en ntered in the acoustic neuromas

is also remarkable that while 100% of the ustic neuroma patients had elevated l fluid protein, only 35% of the 'non romas had this elevation

s regards mortality, the operative results about the same in the two groups, though iring in mind that the malignant tumours are cause of 50% of the deaths Postopera e facial palsy was much less frequent in s group of "non neuromas" of the cere llopontine angle

ACKNOWLEDGEMENT

e histological diagnoses have been made by the Neuro hological institute University of Copenhagen

ZUSAMMENFASSUNG

Symptome praoperative diagnostische Ergebnisse vie histologische Befunde bei 34 Kleinhirnbrucken nkeltumoren – mit Ausnahme von Acusticusneuri men – werden vorgelegt Die Zahl der Meningeome in dieser Gruppe weit uberwiegend Das Symptom ister ist viel weniger gleichartig als bei den Acusticus unnomen wo der 8 Nerv dominiert Der Nervus ialis ist weniger involviert wahrend die übrigen Scha lnerven haufiger Symptome zeigen Ein bemerkens rtes Zeichen ist das Vorkommen von schweren Trige nusneuralgien was wir bei Acusticusneurinom Patienten nicht gefunden haben Von den Nicht Neurinomen hatten nur 35% einen erhohten Proteininhalt in der Spinalflüssigkeit gegen 100% der Acusticusneurinomen Außerdem werden 7 Patienten mit beiderseitigem Acusti cusneurinom erwahnt Die Verwandtschaft mit von Recklinghausens Krankheit und miltiplen Meningeomen wird besprochen

REFERENCES

Boberg Ans J 1955 Clinical examination of corneal sensitivity *Br J Ophthalmol 39* 705

Castellano F & Ruggiero G 1953 Meningeomas of the posterior fossa *Acta Radiol* (Stockh) Suppl *104*

Cushing H & Eisenhardt L 1939 *Meningeomas Their Classification Regional Behaviour Life History and Surgical End Results* Ch C Thomas Springfield Ill

Edvard M & Passy V 1972 Von Recklinghausen s disease with multiple meningeomas *Laryngoscope 82* 2222

Gardner W J & Turner O A 1940 Familial involve ment of the NS by multiple tumors of the sheaths and enveloping membranes *Arch Neurol Psychol 44* 76

Glass B & Abbott K H 1953 Intracranial tumors in siblings *Bull Los Angeles Neurol Soc 18* 46

Hitselberger W E & Gardner G 1968 Other tumors of the cerebellopontine angle *Arch Otolaryngol 88* 712

Nager G T 1964 Association of bilateral VIIIth nerve tumors with meningeomas in von Recklinghausen s disease *Laryngoscope 74* 1220

— 1967 Gliomas involving the temporal bone Clinical and pathological aspects *Laryngoscope 77* 454

von Recklinghausen F 1882 *Über die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen* Berlin

Thomsen J 1975 Suboccipital removal of acoustic neuromas Results of 125 operations *Acta Otolaryngol* (Stockh) *81* 406

Thomsen J & Jørgensen M B 1973 Undiagnosed acoustic neurinomas A presentation of 4 cases *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 204* 175

Thomsen J Lester J & Zilstorff K 1976 Cerebello-pontine Pantopaque cisternography and tomography of the internal acoustic meatus in the diagnosis of angle tumors *Acta Otolaryngol* (Stockh) (in press)

Thomsen J & Terkildsen K 1976 Audiological findings in 125 cases of acoustic neuromas *Acta Otolaryngol* (Stockh) *80* 353

Thomsen J & Zilstorff K 1975 Intermedius nerve involvement and testing in acoustic neuromas *Acta Otolaryngol* (Stockh) *80* 276

Zülch K J 1957 *Brain tumors Their Biology and Pathology* Springer New York

J Thomsen MD
University ENT Dept
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen
Denmark

Acta Otolaryngol 82 112–117, 1976

QUANTIFICATION OF VESTIBULAR DYSRHYTHMIA

N G Henriksson, O Pahlm, P O Borjesson and A I Dahlen

From the Otoneurologic Division, Department of Otolaryngology, University of Lund and the Telecommunication Theory Division, Department of Electrical Engineering, Institute of Technology, Lund, Sweden

(Received November 13, 1975)

Abstract. Quantification of dysrhythmia in a nystagmus response has hitherto not been seriously attempted, possibly because the nystagmus response does not easily lend itself to description in mathematical or physical terms. Ideally the nystagmus response consists of a sequence of triangles. Each such triangle is determined by four qualities, i.e. the velocities and the durations of the fast and slow components. Thus, without serious loss of information it is possible to represent a nystagmus reaction by four sequences of values, one for each of the four above mentioned qualities. From each such sequence we have extracted relevant information and presented it in graphical form. It may be assumed that each of the four nystagmus qualities reflects the activity within separate neuronal systems. Each of these four systems is then informed upon by three parameters for each nystagmus quality, i.e. by its mean, by its variation coefficient and by its sequential variation coefficient. It is claimed that these three diagrams contain nystagmus information not earlier considered.

INTRODUCTORY ANALYSIS

A nystagmus beat in the horizontal plane is determined by four qualities, viz. the velocity of slow (V_1) and fast (V_2) components with their durations (T_1, T_2) (Fig. 1).

A fifth component, the initial position of the eyes, may be added. In this study, however, it will not be considered. Four different series of values, one series for each of these four qualities will then be available in any sequence of nystagmus beats. In any such series x_k, $k=1$, ..., N, the statistical spread (S) can be calculated. By dividing S by the mean of the series the variation coefficient S/M can be achieved

$$\frac{S}{M}=\frac{1}{M}\sqrt{\frac{1}{N-1}\sum_{k=1}^{N}(x_k-M)^2} \quad (1)$$

This expression does not reflect to what extent any value is related to the preceding or the following value in the series. This relationship between consecutive values can, however, be reflected by a sequential variation coefficient $S\Delta/M$

$$\frac{S\Delta}{M}=\frac{1}{M}\sqrt{\frac{1}{N-1}\sum_{k=1}^{N-1}\frac{(x_{k+1}-x_k)^2}{2}} \quad (2)$$

A nystagmus curve with large differences between qualities in different beats inde

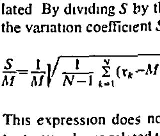
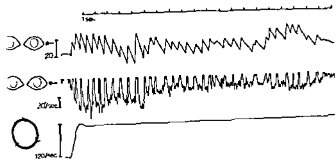


Fig. 1. Four nystagmus qualities determine the nystagmus beats. V_1=speed of slow component, V_2=speed of fast component, T_1=duration of slow, and T_2=duration of fast component.

This paper was presented at "The Brain Conference", Tokushima University, Japan, October 24, 1975.



Fi[illegible] 2 The normal nystagmus rea[illegible] analysed in the present study [illegible]ere [illegible] a p[illegible]p[illegible]r fed at 10 mm/sec

[illegible]dent of sequential position will then pro[illegible]e a large S/M When in such a series large [illegible]lues are found together with other large [illegible]lues and small with other small values this [illegible]oothness will be reflected by a small [illegible]quential variation coefficient $S\Delta/M$. With [illegible]all and large values alternating in con[illegible]utive nystagmus beats this irregularity of [illegible] sequence will be expressed by a large [illegible]/M

These two expressions for stability of the [illegible]stagmus curve, the variation coefficient [illegible]/M and the sequential variation coefficient [illegible]/M, must, in order to be meaningful, refer [illegible] situations with a constant vestibular stim[illegible]is

In a post rotatory nystagmus reaction there [illegible] an exponential decay of velocity of slow [illegible]ases with related variations also of other [illegible]stagmus qualities These variations reflect [illegible] decay in peripheral stimulus and are of [illegible]interest in this connection Any such series [illegible] exponentially varying values may, how[illegible]r, be changed in such a way that the expo[illegible]ial variation is compensated for The re[illegible]ting series may then be regarded as effect [illegible] a constant vestibular stimulus The remain[illegible] variation expressed by the variation coef[illegible]ient S/M and the sequential variation coef[illegible]ient is then mainly due to the dynamic and [illegible]dom properties of the vestibulo-ocular arc

It is now the aim of this paper to describe [illegible]echnique to estimate and display in a post [illegible]otory nystagmus reaction the means of each [illegible] the four different nystagmus qualities with [illegible]ir variation coefficients (S/M) and their [illegible]quential variation coefficients ($S\Delta/M$)

METHODS

In a conventi[illegible]l ro[illegible] [illegible]n chair a[illegible] [illegible]ject, 24 years o[illegible] [illegible] [illegible]xposed to acceleration of 6[illegible] [illegible]se[illegible] [illegible]ec

Registration tech[illegible] [illegible] measurements

The nystagmus was reco[illegible] [illegible] [illegible] a [illegible] [illegible]nique, the signals were [illegible] [illegible] Packard 3960 four chan[illegible]el recorder [illegible] nystagmus signal was th[illegible] [illegible] played Mingograph with the recording paper fed [illegible] cm/sec and with a scale factor of 20° = 1[illegible] From this recording were calculated veloci[illegible] and duration of slow and fast components

Computer processing of [illegible]alues

The four different series of values were fed into a computer For each such series the computer makes an estimate of the initial value and the exponential parameter The original sequence is then multiplied by exponential factors in such a way that the initial value of the resulting sequence is the same as for the original one but the exponentiality is annihilated

For each nystagmus quality were then determined the mean, the variation coefficient S/M and the sequential variation coefficient $S\Delta/M$ This was done before as well as after compensation for exponentiality

RESULT

In Fig 2 [illegible] the post rotatory nystagmus [illegible]d, here with the paper fed

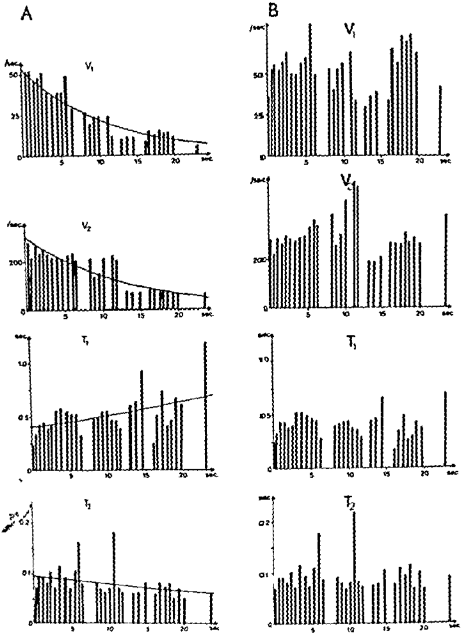


Fig 3 The four nystagmus qualities V_1 and V_2 = speed of slow and fast components T_1 and T_2 = durations for these components here plotted as functions of time—the sequence to the left (A) are values before compensation for exponentiality and to the right (B) after such com-

at 10 mm/sec instead of 100 mm/sec in the display used for calculation

In Fig 3 A the numerical values of each of the four qualities in this post rotatory nystagmus reaction are plotted against time In Fig 3 B the same parameters of the same nystagmus reactions are plotted after compensation for exponentiality

In Table I are presented for all the four qualities studied the initial values the means the variation coefficients *S/M* and the sequen

tial variation
fore and after

In the three
ter compensation
mated means, *M*
ficients, *S/M*
coefficients, *S*
nystagmus
ues related to
nents are p
velocities of

ble I *Initial value (I), mean (M) S/M and SΔ/M of the four different nystagmus qualities—ed of slow component V_1 speed of fast component V_2 duration of slow component T_1 and ration of fast component T_2—before and after compensation for exponentiality in a post y nystagmus reaction*

	Nystagmus qualities							
	Before compensation				After compensation			
	I	M	S/M	SΔ/M	I	M	S/M	SΔ/M
°/sec	53	20	62	17	53	53	26	18
°/sec	306	158	60	29	306	306	37	30
sec	0 41	0 53	56	43	0 41	0 41	38	34
sec	0 08	0 097	38	37	0 08	0 097	35	37

xis, to the durations of slow components on itive Y axis and to the durations of fast omponents on negative Y axis

It is apparent that the speed of slow com has the largest stability of the four alities with the smallest variation coef icient

DISCUSSION

ffect of compensation for exponentiality

t is quite clear from Table I that the variation t for speed of slow component be smaller after compensation for expo- y This is of course to be expected as ow the values are made much more even the exponential variation excluded The that the variation coefficients S/M also the other qualities, duration of slow com t as well as velocity and duration of fast are smaller after this compensa had taken place indicates that also all hese other qualities vary, at least to some ex ent, with peripheral stimulus No further alysis was made with the respect to this

The estimated average nystagmus beat

The values of the four different qualities pre in the upper diagram of Fig 4 de a nystagmus beat representative of particular nystagmus response The calcu of this beat has been based upon values which the exponentiality has been com ed for Note that the area of each rectangle represents an estimate of the aver age nystagmus amplitude after compensation (velocity × duration = amplitude) The differ ence in shape of the fast and slow phase rectangle is apparent A variation in shape of these rectangles may also reveal what quality in the nystagmus beat that might be re- sponsible for a variation in amplitude of nystagmus in different nystagmus responses

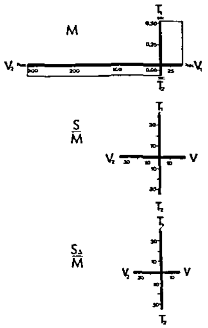


Fig 4 A diagramatic representation of estimated means (M) of variation coefficients (S/M) and of sequential variation coefficients (SΔ/M) for the four different nystag mus qualities velocities V and V_2 durations T_1 and T_2 for slow and fast nystagmus components

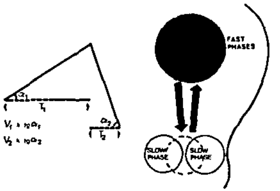


Fig 5 Fast phase (saccadic) system and slow phase system are continuously controlling the corresponding velocities but also constantly interacting with the respect to duration of each phase Each of the four nystagmus qualities may then be reflected by a corresponding system in the brain stem Three types of information is available for each of these systems by determining the mean the variation coefficient and the sequential variation coefficient of a nystagmus reaction

The variation coefficient S/M (after compensation)

The variation coefficients within the series of each of the four nystagmus qualities is displayed in the next diagram of Fig 4 This then provides a comparison between the different qualities with respect to variation but respect to position in the sequence

This spread seems to be smallest for the velocity of the slow components The relative stability of this component may be expected as it must be regarded as meaningsful with a speed of slow component with few and small random variations The main task of the vestibular reflex arc must be to secure proper velocity of the eyes during the slow components allowing visual information to be picked up A variation or a defect in this production of constant velocity would make the whole process of the vestibulo-ocular arc meaningless

Sequential variation coefficient SΔ/M (after compensation)

With a small *S/M* a small spread the sequential variation coefficient will also stay small and may not provide further information With a large *S/M* there are, however two possibilities *SΔ/M* may be large or small

A small *SΔ/M* could then indicate a nystagmus curve with sequences of large values briskly changed into sequences of small values, a pattern described by Bergstedt (1973) It could just as well indicate a sequence of smoothly varying values, although containing large as well as small ones (e g smooth rise and fall type of behaviour) A large value of *SΔ/M* would imply a large variation between the consecutive nystagmus beats and thus an even more irregular nystagmus pattern

The four nystagmus qualities, velocity and duration of slow and fast components, may each be reflected in four different working principles in the brain stem as can be seen in *the model of Fig 5 The velocity of the slow* component should be related to a slow phase system in the brain stem and the velocity of the fast component to a saccadic, fast component system (the pontine para median gaze centre) The durations of these velocities should be dependent upon a reciprocal interaction between the slow and fast systems The duration of the fast component should be dependent upon the ability of this center to maintain its activity, the duration of the slow component of its ability to maintain its velocity

Each of these four nystagmus qualities may then be represented by four different neuronal systems in the brain stem On each of these systems can be informed by three features in the nystagmogram

1 The estimated *mean of the* nystagmus quality
2 The corresponding variation coefficient (*S/M*)
3 The corresponding sequential variation coefficient (*SΔ/M*)

It is hopefully believed that this type of analysis and of display should be useful for interpreting nystagmus responses in various types of experiments as well as in clinical work It will be the aim of future papers of ours to study such diagrams in various types of patients but also in subjects under various loads, antihistaminics, barbiturates, etc It

hould be pointed out, though, that the in ormation derived is not necessarily exhaus ve, and that it may be fruitful—at least in ome cases—also to study higher order sta stical properties

CONCLUSION

our qualities determine the nystagmus beat he velocities and the durations of slow and ast components In a post rotatory nystagmus eaction these qualities will vary between dif erent nystagmus beats partially due to the xponential returning of the deviated cupula The exponential variation between nystagmus ats can be annihilated by a computer, thus roducing a sequence of values without such ariation

The aim of this paper has been to deter- ne and display the remaining variation, the lysrhythmia, between the different nystagmus eats after this exponential variation has been ompensated for

By a computer technique can be achieved our series of values, one for each of the four ualities and with exponential variation an ihilated For all these four series the follow ng three parameters were determined

1 The mean

The statistical spread divided by the mean (variation coefficient)

The sequential spread divided by the mean (sequential variation coefficient)

The result of this analysis can be displayed in three diagrams one for the means one for he variation coefficients and one for the se uential variation coefficients of the four nystagmus qualities studied From these dia rams it is apparent that the variation coef icient and the sequential variation coefficient vere smaller for the speed of slow compo ient than for the other nystagmus qualities ndicating that this quality is the most stable ne in a sequence of nystagmus beats

It is also hopefully assumed that this sys tem for analysis and display of nystagmus responses should be used for various types of vestibular studies This technique may reveal various disorders but also effects of various loads, drugs etc on the vestibular reflex arc

ZUSAMMENFASSUNG

Die quantitative Bestimmung von Nystagmusreaktionen ist bis heute wenig im Angriff genommen worden wahr scheinlich aufgrund der mathematischen und physika lischen Schwierigkeiten die so eine Beschreibung mit sich bringen Idealisch gesehen ist die Nystagmusreak tion eine Reihe von Dreiecken Jedes Dreieck wird durch vier Qualitaten bestimmt das heisst die Geschwindigkeit und die Dauer vom langsamen und schnellen Phase der Nystagmusreaktion Man kann aber ohne grosse Infor mationsverluste vier Reihen von Werten aufstellen eine fur jede der oben erwahnten Qualitäten Von jeder Reihe haben wir die relevante Information extrahiert und in graphischer Form dargestellt Es kann angenommen wer den dass jede der vier Nystagmusqualitaten die Aktivität innerhalb separate neuronale Systems darstellen Bei jedem dieser vier Systeme bekommt man für jede Nystag musqualitat Information durch drei Parameter das heisst durch den Mittelwert durch den Variationskoeffizient und schliesslich durch den sequentiale Variationskoeffi zient Man bekommt durch diese Methoden eine Nystag musinformation die fruher nicht berucksichtigt worden ist

REFERENCES

Bergstedt M 1973 Stepwise change of amplitude and frequency of vestibular nystagmus *Adv Otorhino-laryngol 19* 304

Blegvad B 1962 Caloric vestibular reaction in uncon scious patients *Arch Otolaryngol 75* 506

Claussen C F 1969 Das Frequenzmaximum des kalo-risch ausgelösten Nystagmus I als Kennlinienfunk tion des gepruften Vestibular organes *Acta Otola ryngol* (Stockh) *67* 639

Clément P A R H E M 1970 *Dysrhythmia during caloric nystagmus* Thesis (Mur Aalsmeer Amst)

Henriksson N G 1955 An electrical method for registra tion and analysis of the movements of the eyes in nystagmus *Acta Otolaryngol* (Stockh) *45* 25

— 1956 Speed of slow component and duration in caloric nystagmus *Acta Otolaryngol* (Stockh) Suppl *125* 1

— 1973 Patterns of eye movements and their clinical evaluation *Japan Equil Res 32*

Lundgren A Tibbling L & Henriksson N G 1969 DC-determined displacement of the nystagmus beat in rotatory tests *Pract Otorhinolaryngol* (Basel) *31* 54

Mittermaier R & Christian W 1954 Dauer Schlag zahl und Gesamtamplitude des kalorischen Nystag mus *Z Laryngol Rhinol 33* 20

Riesco-McClure J & Stroud M 1960 Dysrhythmia in the post-caloric nystagmus Its clinical significance *Laryngoscope 70* 697

Torok N 1948 Significance of the frequency in caloric nystagmus *Acta Otolaryngol* (Stockh) *36* 38

N G Henriksson M D
Dept of Otolaryngology
University of Lund
S 221 85 Lund Sweden

Acta Otolaryngol 82 118–122, 1976

ENDONASAL VENULAR PERMEABILITY IN RATS EXPOSED TO THE COLD

M W Kanan and P van Diest

From the Department of Human Anatomy The United Oxford Hospitals Oxford England

(Received May 10 1975)

Abstract Sixteen male rats were killed 22 hrs after being injected with a dose of 4 mCi ^{198}Au mixed with 16 mg of inert colloidal gold/100 g body wt The anterior nasal mucosa of the septum and turbinates in 10 rats which were injected and kept in a temperature of 5–7°C showed mean radioactive counts 2–3 times higher than those recorded in the same areas in 6 control rats kept in ordinary room temperature (22°C) In all sixteen rats the highest counts were obtained in the RES organs namely the liver spleen adrenals and the bone marrow in descending order Other tissues revealed no significant radioactivity except for minor counts in the lungs and kidneys The importance of these findings regarding the localisation of granulomatous diseases and vasculitis in nose is discussed

A recent study of the behaviour of various tissues of experimental animals towards a single intravenous dose of 8 or 16 mg of colloidal carbon (Kanan et al 1975, in press), significant uptake of the carbon occurred in the lamina propria of the anterior nasal septum and turbinates The authors also observed enhanced leakage of the carbon through the walls of those venular capillaries facing the inspired air stream in animals exposed to the cold The following is a report of a quantitative study of the distribution of a comparable single intravenous dose of ^{198}Au in the tissues of male rats exposed to a cold atmosphere

MATERIALS AND METHODS

Sixteen male Wistar rats of 220 g average wt fed on laboratory chow and water ad libitum were used for this experiment Six of them were kept at room temperature (22°C) during the intravenous injection of ^{198}AU and until the time of killing 22 hrs later Another batch of 10 rats were exposed to an atmospheric temperature of 5–7°C in an air conditioned room for 6 hours before, during the injection and until the time of killing 22 hrs later

A radioactive gold suspension ^{198}Au obtained from Amersham Radiochemicals was mixed with a suspension of inert colloidal gold (Amersham Radiochemicals, UK) of particle size 20–30 nm so that the mixture contained 5 mCi ^{198}Au + 20 mg inert colloidal gold/ml Each animal was injected intravenously with a dose of 16 mg inert colloidal gold + 4 mCi of ^{198}Au/100 g body wt The animals were anaesthetised with an intraperitoneal injection of 2% tribromoethanol Avertin solution (Bayer Products Co, Surbiton Surrey, UK) at dose of 0 1 ml/100 g body wt The gold sol mixture was administered into the external jugular vein of the rat through a skin incision

All rats were killed 22 hrs after the gold injection with intraperitoneal veterinary Nembutal (Abbott Laboratories) and tissues were obtained from various areas of the skin mucosa of the nose mouth and eyelid and from the internal organs as indicated in Tables I and II

Every piece of tissue was weighed, placed deep down into an LP3 tube which was

This work has been supported by an M R C grant (G973/712 T)

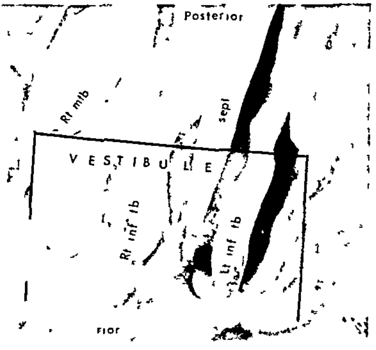


Fig 1 *Rt mtb*=right middle turbinate or nasoturbinal *Inf tb*=inferior turbinate or maxilloturbinal *Rt*=right *Lt*=left *sept* nasal septum

ven a serial number and a tissue code prior counting Six control empty LP3 tubes ere fed together with all other tubes contain g the tissue fragments into a Nuclear hicago Autogamma Counter set for 1 minute ounting per tube

The mean background radioactivity re orded for the empty tubes was deducted om every count obtained for each piece of sue and the resulting figure was corrected correspond to 100 mg of tissue The pieces tissue obtained from the nasal cavity re d in Table I correspond to the areas esignated in Figs 1 and 2

RESULTS

hese are summarised in Tables I and II mean values of the radioactive counts tained per 100 mg of tissue in the retro-stibular anterodorsal part of the nasal m and the naso and maxilloturbinals om all the animals were significantly higher an those recorded for other areas of the skin and mouth On the other hand the values of the counts recorded for the e areas of the nasal mucosa in the rats exposed to an atmospheric temperature of 5–7°C were two to three times higher than the figures obtained in the corresponding areas in the control animals kept at 22°C (Table I) However exposure of animals to the cold seemed to produce some increase in the radioisotope uptake by the ear and scrotum but not by other tissues outside the nasal mucosa

Among the members of the RES the mean counts recorded for the liver were 8–10 times higher than those recorded for the spleen In the adrenals and bone marrow the mean counts were 5–7 times lower than in the spleen

Random samples from the blood and other tissues showed very low counts except for

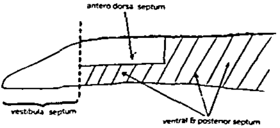


Fig 2 Biopsy diagram—side view

Table I *Distribution of intravenous* ^{198}*Au in male Wistar rats after 22 hours*

Tissue 100 mg	Control animals (22°C) 1	2	3	4	5	6	Mean value (cpm)	Cold-exposed (5–7°C) rats 1	2	3	4	5	6	7	8	9	10	Mean value (cpm)
Nose tip	5	2	2	0	0	3	2	6	2	4	0	0	0	0	2	0	4	1 8
Nasal vestibule	9	12	8	0	0	2	5	16	12	5	13	15	11	15	8	12	10	11 7
Anterior dorsal septum	154	140	165	138	152	149	150	405	330	560	289	362	290	300	311	390	355	359
Posterior & ventral septum	23	22	36	28	19	30	26	70	62	75	90	80	97	100	95	82	78	81
Middle turbinate	130	128	129	134	133	132	131	250	262	267	239	270	270	233	222	235	225	247
Inferior turbinate	138	130	142	140	136	132	136	260	289	252	282	230	242	290	269	300	271	268
Lip	2	0	6	0	0	2	1 6	8	6	8	2	0	0	4	4	4	0	3 6
Tongue	0	0	0	0	2	4	1	0	0	0	0	6	2	0	2	0	0	1
Ear	18	19	15	20	22	20	19	32	35	30	28	28	22	34	30	36	32	30 7
Footpad	0	0	0	0	4	2	1	8	14	12	10	9	2	6	9	12	10	9 2
Scrotum	0	0	0	4	8	0	2	26	19	20	22	20	18	12	15	12	15	18
Buccal mucosa	10	6	6	0	0	4	4	6	0	2	4	12	0	0	10	8	4	4 6

the lung and kidney which recorded slightly significance means

Apart from the nasal mucosa, exposure of animals to cold did not seem to influence the tissue distribution of ^{198}Au to any significant degree

DISCUSSION

A mixture of tracer radiogold with inert colloidal gold sol has been considered most suitable for the quantitative study of the granulopectic activity of the RES (Barrow et al , 1951, Sheppard et al , 1947*a*) It has also been thought ideal for therapeutic use i humans with diseases of the lymphoid systei (Sheppard et al , 1947*b*) This opinion wa based on the relative intoxicity, lack of ant genicity, stability and the uniformity of th injectable particles together with amenabilit to quantitative assay both *in vivo* and in ex cised tissues The half life of ^{198}Au is 2 7 days but there was no need in the preser experiment to plot a radioactivity decay curv since all the tissues were counted simul taneously However, the findings in the pre sent study support the pattern of distributio of radioactive gold in the main organs of th

Table II *Distribution of intravenous* ^{198}*Au in male Wistar rats after 22 hours*

Tissue 100 mg	Control animals (22°C) 1	2	3	4	5	6	Mean value (cpm)	Cold-exposed (5–7°C) rats 1	2	3	4
Liver	30 753	32 100	34 200	35 000	34 200	32 500	33 125	30 090	34 210	35 150	32 4
Spleen	4 428	3 875	3 982	2 976	3 442	3 640	3 724	4 481	2 875	4 673	4 2
Bone marrow		540	536			670	582	–	670		
Adrenals	740	751				674	722		820		
Lung	116	104	126				115	–	140		
Testis	9		6		5		6 6	–	8		
Kidney	60		46			57	54	–	53		
Blood	13		10			15	12 6	–	9		
Synovium	0		0			0	0		–		
Thymus	27		32			17	25	–	12		
Salivary gland			42			32	37	39	–		

S as reported by Barrow et al (1951) ke that reported by Dobson et al (1949) latter authors reported higher distribution olloids with small particle size in the bone w and spleen than in the liver

e present findings confirm quantitatively nt observations made on the nasal mucosa ig intravenous colloidal carbon (Kanan et in press) In the latter study, carbon icles were shown to be phagocytosed by endothelial cells lining the venular capil-s and venules and by the pericytes and ivascular macrophages in the anterior nasal osa Leakage of intravenously ad-istered carbon particles through the inter-ular junctions of the venular endothelium the porous basement membrane on the minal surface of these vessels seemed to enhanced by exposure of the injected ani-s to atmospheric cold

n an ultrastructural study of the nasal cosa, Cauna & Hinderer (1969, 1970) cribed endothelial fenestrations and ous basement membrane on the venular of the microcirculation in the superficial na propria They speculated that these sels were designed for the rapid passage fluids and solutes from the microcircula to the secretory epithelium and vice sa On the other hand, Kanan et al (in ss) expressed the opinion that the peculiar osity of the venular capillaries and the

6	7	8	9	10	Mean value (cpm)
33 009	32 260	29 108	34 500	37 012	32 994
3 212	3 010	2 920	4 100	3 612	3 671
	412				514
			755		745
			120		137
		2	3	2	4 7
		24	38		37
		11		5	7 7
		0		0	0
		26		14	17 1
		–		–	35

vascular basement membrane nur ir venules in the mammalian nasal muc partly caused by the relative stasis c from chronic bombardment of the nasal mucosa by the cool front of spired air stream in postnatal life

Apart from the phagocytotic capacity the specific structure of the RES and the described areas of the nasal mucosa other factors have to be considered in the process of phagocytosis Following the advent of particular matter into the circulation immediate aggregation of thrombocytes occurs in most areas of the microcirculation (Tait & Elvidge, 1926, Salvidio & Crosby, 1960, Stehbens & Florey, 1960, Cohen et al, 1965, French, 1967, van Aken et al, 1968) In organs with high fibrinolytic activity such as the lung platelets soon disaggregate in the microcirculation RES sites with low fibrinolytic activity, particularly the liver and spleen have a lower capacity for platelet disaggregation hence accumulation of blood borne particles enhances phagocytosis of such particles by the macrophages lining their sinusoids (van Aken & Vreeken, 1969)

As demonstrated by Jensen et al (1953) exposure of rats and humans to cold causes depression of the fibrinolytic activity of their blood It is conceivable therefore that the exposure of the nasal mucosa to a cold environment would impede the disaggregation of platelets and thus enhance the deposition of blood borne particles in the catchment area of septum and turbinates The influence of atmospheric cold on the leakage of blood-borne particles is probably relevant to the predilective initial localisation of granuloma producing microorganisms and immune complexes of vasculitis in the anterior nasal mucosa (Kanan & Ryan, 1975)

Moreover, there is increasing evidence that the pathogenic effects of such noxious agents is connected with the depressed cell mediated immunity in areas of the body with temperature cooler than the normal body temperature

ACKNOWLEDGEMENT

We extend our thanks to Professor A G M Weddell and Dr G Fink of the Human Anatomy Department Oxford University for their help and to Dr Ross Munro of the Department of Radiophysics Churchill Hospital Oxford for his valuable advice

ZUSAMMENFASSUNG

Von insgesamt 16 männlichen Ratten wurde 22 Stunden nach Injektion von Au[98] (Dosis 4 mCi in 16 mg unmarkiertem Goldsol/100 g Korpergewicht) die Nasenschleimhaut des vorderen Septumbereiches sowie der Muscheln entnommen und deren Radioaktivitat bestimmt Dabei zeigten die Schleimhaute jener Ratten ($n=10$) die bei einer Temperature von 5–7°C gehalten worden waren ein im Mittel 2–3mal höheren Zahlwert als er in der Mukosa von Tieren ($n=6$) gefunden wurde die bei ublicher Raumtemperatur (22°C) gehalten wurden Bei samtlichen Tieren ($n=16$) wurden die hochsten Radioaktivitaten in den zum RES gehörenden Organen festgestellt und zwar mit sinkender Tendenz in Leber Milz Nebennieren und Knochenmark Mit Ausnahme einer geringen Aktivitat in Lungen und Nieren zeigten die ubrigen Gewebe dagegen keine biologisch ins Gewicht fallende Strahlung Die Bedeutung dieser Ergebnisse fur die Lokalisation von Granulationsgewebe und Vaskulitiden in Nasenbereich wird diskutiert

REFERENCES

Barrow J Tullis J L & Chambers F W 1951 Effect of x radiation and antihistamine drugs on the reticulo-endothelial system measured with colloidal radiogold *Am J Physiol 164* 822

Cauna N 1970 Electron microscopy of the nasal vascular bed and its nerve supply *Ann Otol Rhinol Laryngol 79* 443

Cauna N & Hinderer K H 1969 Fine structure of blood vessels of the human nasal respiratory mucosa *Ann Otol Rhinol Laryngol 78* 865

Cohen P Braunwald J & Gardner F H 1965 Destruction of canine and rabbit platelets following intravenous administration of carbon particles or endotoxin *J Lab Clin Med 66* 263

Dobson E L Gofman J W Jones B Kelly L S & Walker L A 1949 Studies with colloids containing radioisotopes of yttrium zirconium columbium and lanthanum *J Lab Clin Med 34* 305

French J E 1967 Blood platelets Morphological studies on their properties and life cycle *Br J Haematol 13* 595

Jensen H Chamovitz D L Volkringer E Gray E James B A Gosney, J E & Kocholaty W 1953 Antifibrinolytic activity in plasma after exposure to cold and after chymotrypsin administration *Blood 8* 324

Kanan M W & Ryan T J 1975 Endonasal localisation of blood borne viable and non viable particulate matter *Br J Dermatol 97* 475

Kanan M W Ryan T J & Weddell A G M 1975 The behaviour of the nasal mucosa and other tissues toward blood borne colloidal carbon in experimental animals *Pathol Eur* in press

Salvidio E & Crosby W H 1960 Thrombocytopenia after intravenous injection of India ink *J Lab Clin Med 56* 711

Sheppard C W Goodell J P B & Hahn P F 1947*a* Colloidal gold containing the radioactive isotope [198]Au in the selective internal radiation therapy of diseases of the lymphoid system *J Lab Clin Med 32* 1437

Sheppard C W Wells E B Hahn P F & Goodell J P B 1947*b* Studies of the distribution of intravenously administered colloidal sols of manganese dioxide and gold in human beings and dogs using radioactive isotopes *J Lab Clin Med 32* 286

Stehbens W E & Florey H W 1960 The behaviour of intravenously injected particles in chambers in rabbits ears *Q J Exp Physiol 45* 252

Tait J & Elvidge A R 1926 Effect upon platelets and on blood coagulation of injecting foreign particles into the blood stream *J Physiol 62* 129

van Aken W G Goote Th M & Vreeken J 1968 [illegible] hanism m [illegible] ulation of macromolecular particles in the reticuloendothelial system (RES) mediated by platelet aggregation and disaggregation *Thromb Diath Haemorrh 22* 496

M W Kanan M.R C P
Dept of Dermatology
The United Oxford Hospitals
The Slade Hospital
Headington
Oxford OX3 7JH
England

Acta Otolaryngol 82 123-130, 1976

IMMUNOGLOBULINS IN MAXILLARY SINUS SECRETION

C Carenfelt, C Lundberg and K Karlén

From the Department of Otolaryngology Karolinska Sukhuset and the Department of Immunology National Bacteriological Laboratory Stockholm Sweden

(Received October 23 1975)

Abstract The immunoglobulin pattern was studied in maxillary sinus secretion of 17 patients with maxillary sinusitis By single radial *immunodiffusion* measurable amounts of IgM were found in the secretions of 15 pa- [illegible]

[illegible] ...ous secretion) were calculated to be locally produced on average For IgG these figures were 15% (purulent se- [illegible]

[illegible] ... of local immunity in regard to bacterial infections

During the past decade a considerable number of investigations have accumulated concerning the mechanisms involved in immunity and resistance to infection Although Bing & Plum suggested in 1937 that serum globulins originated from plasma cells and although Walsh & Cannon suggested *in 1938* a local production of antibodies in nasal mucosa, the theory of transfer of antibodies from serum through the mucous membranes to external *secretions* (i e secretions that bathe mucous *membranes* having direct contact with *the external* environment) was widespread However, the finding of a predominance of IgA in external secretions as opposed *to serum* (Hanson, 1961 Tomasi & Ziegelbaum, 1963), *indicated* a local production of this immunoglobulin The discovery of antibodies in external secretion produced locally by the mucous membrane has given rise to a new dimension in our understanding of the biological defence mechanism Although most investigations have focused on the presence of the locally produced form of IgA—the secretory IgA (SIgA)—IgG and IgM are also *present* in external secretions and not only as a consequence of "leakage" from serum Thus, there is evidence indicating a local synthesis of IgG and IgM as well (*Brandtzaeg et al* , *1967*, Donovan et al , 1970)

The pattern of immunoglobulins in external secretions such as colostrum, saliva and gastrointestinal fluids has been studied extensively (Tomasi & Bienenstock, 1968) *In the respiratory* tract, *bronchial secretion* (Keimowitz, 1964, Deuschl & Johansson, 1974) and *nasal secretion* (Remington et al , 1964, Lorin et al , 1972) have *been* investigated, and recently also *middle* ear effusion (Van de Calseyde et al , 1971, Mogi & Honjo, 1972, Bernstein et al , 1974)

This study was undertaken to determine the *concentration* of IgM, IgA and IgG in secretion *retained* in maxillary sinus It was also considered of interest to investigate the immunoglobulin content in purulent secretions containing large numbers of bacteria and inflammatory *cells* in comparison with serous secretions where the numbers of bacteria and inflammatory cells were sparse. A further in-

tention was to estimate the amounts of locally produced immunoglobulins in purulent and serous maxillary sinus secretions

MATERIAL AND METHODS

Patients

Seventeen untreated patients (13 female and 4 male, age range 21–61 years) with symptoms of maxillary sinusitis for 3–30 days and with retained secretion in sinus maxillaris, were selected for the present study Samples of sinus secretion were obtained by aspiration through a Lichwitz cannula inserted into the maxillary sinus through the inferior nasal meatus Purulent secretion was obtained from 10 patients, and serious secretion from 7 patients Secretions mainly of low viscosity, opaque and discoloured were designated as purulent The oxygen tension (PO_2) measured in these secretions was always zero, or almost so (<10 mmHg) The clear, pale yellow secretions of low viscosity, frequently with the capacity to gel, were classified as serous In ese secretions PO_2 was always above mmHg (Carenfelt & Lundberg, to be published) Specimens macroscopically tinged with blood were discarded Samples of venous blood for serum were taken simultaneously The secretions and serum were stored at −20°C until analysed

Bacteriological methods

Each sample aspirated from the maxillary sinus was immediately inoculated in broth agar bottles, prepared by the National Bacteriological Laboratory, one for aerobic and one for anaerobic growth Furthermore swabs dipped in the aspirated secretion were inserted into a transport substrate tube (Loeffler) which together with the bottles were handled within 12 hours in accordance with the routine procedures of the Department of Microbiology Karolinska sjukhuset Direct smears from all secretions were stained by Gram s method The number of bacteria was estimated semi quantitatively In some cases, the bacterial and cellular counts were made immediately in a Bürker chamber

Immunological methods

The stored secretions were diluted 1 2 to 1 4 in phosphate-buffered saline (PBS) and homogenized by gentle shaking The supernatant after centrifugation at 3 000 rpm for 20 minutes was used for protein determination The concentrations of IgM, IgA, IgG and albumin in the diluted secretions and serum were determined by the single radial immunodiffusion method essentially as described by Mancini et al (1965) For the quantitation of IgA, a serum IgA (7 S) was used as standard The protein concentrations of serum and diluted secretions were determined on two separate occasions The analytical errors found were, for serum IgM 6%, IgA 4%, IgG 5%, albumin 5%, and for secretion IgM 8%, IgA 6%, IgG 5%, albumin 9%

To evaluate the capacity of IgA to become specifically or non specifically bound to bacteria and inflammatory cells, 14 of the secretions were diluted in 0 1 M glycin HCl, pH 2 6 and homogenized by gentle shaking in order to split up these bindings The hydrogen ion concentration was adjusted to pH 2 3–2 7 with 0 1 M HCl The final dilution was then 1 4 to 1 7 After centrifugation at 3 000 rpm for 20 minutes, the supernatant was neutralized (pH 7 0–7 4) within 45 minutes using Trizma Base (Sigma, St Louis Mo) and used for protein determination as above

Albumin was chosen as an inert reference protein since the synthesis of albumin in the mucous membrane of maxillary sinus is highly improbable Thus, the ratio of secretion albumin (Alb_{secr}) to serum albumin (Alb_{ser}) was assumed to reflect the passage through the mucous membrane, not only of albumin but also of serum immunoglobulins (Ig_{ser}) despite the differences in molecular weights The locally produced immunoglobulin (LP) can then be calculated according to the formula (Deuschl

& Johansson, 1974) $LP = Ig_{secr} - Alb_{secr} / Alb_{ser} \times Ig_{ser}$, where Ig_{secr} is the total amount of the secretion immunoglobulin

Statistical methods

The analytical errors were calculated by the formula

$$S\,D = \sqrt{\frac{\left(\frac{d}{\bar{x}}\right)^2}{2n}}$$

where d is the difference between the two determinations, $\bar{x}$ is the mean value of the two determinations and n is the number of duplicate determinations For the statistical analysis of mean protein concentration Student's t test was used For the analysis of mean protein ratios, nonparametric statistical methods were used (Dixon & Massey, 1957)

Table I *Secretions from maxillary sinus specified to bacterial growth and PO_2*

Patient no	Bacterial growth	PO_2 (mmHg)
Purulent secretion		
1	*H influenzae*	0
2	Pneumococcus	0
3	*H influenzae*	–
4	Pneumococcus	0
5	Pneumococcus	0
6	Pneumococcus	2
7	*H influenzae*[a]	0
8	Pneumococcus	2
9	Pneumococcus	0
10	*H influenzae*	0
Serous secretion		
1	No growth	114
2	No growth	65
3	Anaerobic organism[b]	–
4	No growth	101
5	No growth	120
6	No growth	–
7	Pneumococcus[b]	62

[a] Mixed flora with *Staphylococcus albus*
[b] No bacteria was found in smears
– Not done

RESULTS

Bacterial findings

Heavy growth of pneumococcus *or H influenzae was obtained in all purulent secretions* and in 9 of these 10 secretions in pure culture (Table I) Large numbers of inflammatory cells were seen The number of bacteria varied from 10/field (800×) in some secretions to >1000/field in others On the other hand, in the serous secretions there was usually no growth However, when growth did occur, no or very few bacteria were seen on smears Furthermore, the number of inflammatory cells was sparse

Immunoglobulin determination

The individual values and the mean concentration of IgM IgA IgG and albumin in purulent sinus secretion and serum from 10 patients are listed in Table II The corresponding values for serous secretion from 7 patients are given in Table III As seen from these tables, the three immunoglobulins were present in all the secretions although only traces of IgM were found in two of the purulent secretions

The mean concentrations of immunoglobulins and albumin were lower in the purulent than in the serous secretions, but only for IgA was the difference statistically significant ($P<0\ 05$) These values refer to the secretions diluted in PBS However, the IgA content was essentially the same when the hydrogen ion concentration was reduced in the secretions

*The quantitative distribution of the im*munoglobulins in secretion was similar to that of serum, although not identical Thus, the mean ratio of IgG/IgA of serous secretion (3 8) (Table III) seemed to be lower than the corresponding serum ratio (5 9) In purulent secretion the mean ratio (4 2) (Table II) was significantly lower than the corresponding serum ratio (5 5) ($P<0\ 05$)

As seen from Table IV, the secretion/serum ratios for IgA and IgG in both groups of secretion were higher than the corresponding albumin ratios, but only the IgA ratios were significantly higher than the albumin ratios (purulent group $P<0\ 01$, serous group $P<0\ 05$)

Table II *Concentrations of immunoglobulins and albumin (alb) in serum and purulent secretion*

The values are given in mg % nm not measurable (traces)

Patient no	Serum				Secretion				IgG/IgA	
	IgM	*IgA*	*IgG*	*Alb*	*IgM*	*IgA*	*IgG*	*Alb*	*Serum*	*Secretion*
1	124	258	1 906	4 346	nm	139	362	373	7 4	2 6
2	62	261	1 307	3 917	24	134	566	1 432	5 0	4 2
3	80	404	1 481	3 955	73	351	1 067	3 245	3 7	3 0
4	112	245	1 480	3 401	155	434	1 909	3 944	6 0	4 4
5	112	285	1 355	4 902	64	193	871	3 153	4 8	4.5
6	193	277	1 328	4 215	120	182	770	2 647	4 8	4 2
7	93	234	1 328	3 787	94	234	1 394	3 291	5 7	6 0
8	142	304	1 379	4 092	122	268	1 067	3 062	4 5	4 0
9	88	191	833	3 197	nm	100	272	556	4 4	2 7
10	101	157	1 357	3 482	52	194	1 196	2 430	8 6	6 2
Mean	111	262	1 375	3 929	(88)	223	947	2 413	5 5	4 2
S D	37	67	260	502	(43)	104	494	1 220	1 4	1 2

In all of 17 secretions, as seen from Table V, the calculations showed the presence of locally produced IgA In 15 of 17 secretions IgG was calculated to be produced locally Statistical analyses of these data showed that the local production of IgA and IgG was significant (IgA $P<0\ 001$, IgG $P<0\ 01$) The calculation of the local production of IgM was pointless because of the uncertainty in the calculation formula for this protein The locally produced IgA was calculated to 56 mg% on average for 10 purulent secretions corresponding to 30% of the total content of IgA in the secretions The same calculation for IgG showed that 105 mg% was locally produced, corresponding to 15% For the group of 7 serous secretions the figures were higher, for IgA 250 mg% and for IgG 301 mg%, corresponding to 43 and 18% respectively The difference in mean concentration of locally produced IgA between the two groups of secretions was statistically significant ($P<0\ 05$)

DISCUSSION

The immunoglobulin content has previously been determined in external respiratory tract secretions during purulent and bacterial infections such as pneumonia, bronchitis (Biberfield & Sterner, 1971, Falk et al, 1972 Deuschl & Johansson 1974) and purulent otitis (Mogi et al 1973) However, in these investigations the results have not been related

Table III *Concentrations of immunoglobulins and albumin (alb) in serum and serous sinus secretion*

The values are given in mg %

Patient no	Serum				Secretion				IgG/IgA	
	IgM	*IgA*	*IgG*	*Alb*	*IgM*	*IgA*	*IgG*	*Alb*	*Serum*	*Secretion*
1	158	112	1 456	3 538	50	260	1 196	2 316	13 0	4 6
2	241	177	1 075	4 795	84	146	636	2 504	6 1	4 4
3	110	530	1 530	3 925	60	1 070	1 620	2 430	2 9	1 6
4	89	350	1 009	4 630	46	220	1 646	2 864	2 9	7 5
5	145	146	1 283	3 997	128	388	1 584	4 302	8 8	4 1
6	180	530	1 905	3 589	180	900	1 425	2 205	3 6	1 6
7	113	307	1 139	3 552	96	358	941	4 058	3 7	2 6
Mean	148	307	1 342	4 004	92	470	1 293	2 954	5 9	3 8
S D	52	174	314	519	48	346	387	865	3 8	2 1

Table IV. *The sinus secretion/serum ratio for each immunoglobulin and albumin (alb)*

Patient no	Purulent secretion/serum				Patient no	Serous secretion/serum			
	IgM	IgA	IgG	Alb		IgM	IgA	IgG	Alb
1	–	0 54	0 19	0 09	1	0 32	2 32	0 82	0 65
2	0 39	0 51	0 43	0 37	2	0 35	0 82	0 59	0 52
3	0 91	0 87	0 72	0 82	3	0 55	1 92	1 06	0 62
4	1.38	1 77	1 29	1 16	4	0 52	0 63	1 63	0 62
5	0 57	0 68	0 64	0 64	5	0 88	2 66	1 23	1 08
6	0 62	0 65	0 58	0 63	6	1 00	1 70	0 75	0 61
7	1 01	1 00	1 05	0 87	7	0 85	1 17	0 83	1 14
8	0 86	0 88	0 77	0 75					
9	–	0 52	0 33	0 17					
10	0 51	1 24	0 88	0 70					
Mean	(0 78)	0 87	0 69	0 62		0 64	1 60	0 99	0 75
S D	(0 32)	0 40	0 33	0 33		0 27	0 76	0 35	0 25

to the bacterial diagnosis, number of bacteria and to the occurrence of inflammatory cells As immunoglobulin deficiencies result in bacterial sinopulmonary infections in particular, it was considered of value to investigate the quantitative immunoglobulin distribution in purulent maxillary sinus secretion

Most studies of external secretions from the respiratory tract have relied upon material obtained by instillation of saline into the bronchi or nasal cavity and recovery of a part of the fluid together with an unknown amount of secretion The procedure implies that no absolute values of concentrations of different components are available, but only concentration ratios In the present study, on the contrary, adequate volumes (1–7 ml) of undiluted secretion were obtained from the maxillary sinus Thus, the concentrations of immunoglobulins could be expressed in absolute values, which is of great advantage in immunological studies Furthermore, as the secretion is easily available to aspiration, specimens can be obtained repeatedly. An other advantage connected with the maxillary sinus is that the healthy antrum is sterile (Bjorkwall, 1950) When infected, the bacterial strains responsible for the majority of these infections are limited In non sterile secretions Rantanen & Arvilommi (1973) found pneumococcus of *H influenzae* to be the dominant bacteria (75%), which is in agreement with our experience Using the aspiration technique, as in this study, instead of the irrigation of sinus, it is also possible to obtain representative specimens with little risk of bacterial contamination from the nasal cavity Thus, the maxillary sinus is a suitable "mod-

Table V *Mean concentration (mg%) of serum derived and locally produced (LP) IgA and IgG and the local production in % of the total content (LP%) of IgA and IgG in purulent secretion of 10 patients and in serous secretion of 7 patients*

Secretion	Serum derived		LP		LP%	
	IgA	IgG	IgA	IgG	IgA	IgG
Purulent						
Mean	167	842	56	105	30	15
Range	22–331	145–1 716	7–150	−148–249	4–84	−14–55
Serous						
Mean	220	992	250	301	43	18
Range	73–351	561–1 381	4–692	−360–1 022	2–72	−38–62

el in the investigation of local immunity in regard to pyogenic infections

As large numbers of bacteria and inflammatory cells might interfere with the evaluation of the immunoglobulin content and its turnover in purulent secretion, it justified a comparison of serous secretion containing a small number of inflammatory cells and bacteria In the purulent secretions, a heavy growth of pneumococcus or *H influenzae* was uniformly cultured It should be noted here that these pathogens are of interest in maxillary sinusitis, not only because of their frequent occurrence, but also because of the therapeutic difficulties associated with these strains (Carenfelt et al, 1975) Objectively the two types of secretion could be distinguished from each other by bacterial growth and the difference in oxygen tension The low oxygen tension in the purulent secretions was due to heavy oxygen consumption by the microorganisms and the inflammatory cells (Carenfelt & Lundberg, to be published)

When aspirating sinus secretion, an admixture of blood could not always be avoided However, those samples macroscopically tinged with blood were discarded The Guaiac test was not utilised, as the remaining samples could be assumed to contain negligible amounts of blood (Biberfield & Sterner, 1971)

In determination of IgA in secretions, the concentrations might have been higher had a SIgA (11 S) preparation instead of IgA (7 S) been used as the standard in the immunodiffusion test However, since the secretion is formed in connection with an inflammatory process, the possibility of local production not only of SIgA but also of monomeric IgA (7 S) without the capacity of binding the secretory component (Brandtzaeg 1974) must be taken into account In addition, a rather large proportion of IgA in the secretions emanated from serum Thus, the assay procedure used here might result in a certain underestimation of IgA in the secretion, though hardly threefold as previously reported (Tomasi & Bienenstock, 1968)

This investigation shows that IgM IgA and IgG are present in human maxillary sinus secretion under pathological conditions The concentration of immunoglobulins and albumin seemed to be lower in the purulent than in the serous secretions The reason for this is not clear Thus, a number of factors of unknown importance may interfere with the turnover of components of the secretions An addition of high molecular proteins from lysed bacteria and from the damaged mucosa may result in an increased colloid osmotic pressure and consequently in a dilution of the purulent secretions Another possible explanation for the difference between the two types of secretion is degradation of proteins by proteolytic enzymes released from inflammatory cells and bacteria As the mean level of IgA was significantly lower in purulent secretion than in serous secretion and as IgA has a great propensity to form complexes with the most diverse kinds of proteins (Heremans et al 1966), we thought it of interest to examine to what extent IgA was adsorbed to bacteria and inflammatory cells and consequently lost to the sediment at centrifugation of the specimens For this purpose the hydrogen ion concentration was reduced in the secretions as IgA adsorbed to bacteria can be eluated in acid environment (Brandtzaeg et al, 1968) However, the IgA content in the secretions handled in this way was unaltered not only in serous secretion, but also in purulent secretions, despite the difference in number of bacteria and inflammatory cells Thus, the possible loss of IgA to the sediment must be small

The concentration ratio IgG/IgA of most external secretions examined has been below 1 During mucosal inflammatory processes the transfer of immunoglobulins to the secretion is enhanced and accordingly the ratio approaches that of serum (about 6 1) (Tomasi & Bienenstock, 1968) Therefore, it was not unexpected to find this concentration ratio above 1 in the sinus secretions Thus, altered permeability of the inflamed mucous membrane is one mechanism explaining the high ratio IgG/IgA in si-

nus secretions Another possible mechanism might be an increased local production of IgG, as an increased local proliferation of IgG immunocytes has been found in inflamed nasal mucosa (Brandtzaeg, 1967)

Differences in permeability of the sinus mucosa and in local production of immunoglobulins might explain the rather wide range of individual values of immunoglobulins in the sinus secretions, although other factors (dilution of secretion, proteolysis) governing the turnover of components in sinus may interfere However, the transmucosal transport process is not known in detail Nor is the role of diffusion, filtration and pinocytos as transvascular transport mechanisms of macromolecules established (Parving et al 1974, Lonsmann Poulsen, 1974) In this connection it is worth noting that the ratio of secretion albumin to serum albumin in this study (0 62–0 75) was of the same magnitude as the ratio of interstitial fluid (whole body) albumin to serum albumin (0 66) (Lonsmann Poulsen 1974)

The present results show that IgA and IgG are locally produced in maxillary sinus As the calculation formula used in the calculation of locally produced IgA and IgG does not take in to consideration that the molecular weight of albumin is less than those of the immuno globulins the calculated values of IgA and IgG were underestimated, although probably to a rather small extent (Donovan et al 1970) However, because of the large difference in molecular weight between albumin and IgM the formula was unsuitable in calculation of locally produced IgM

In conclusion, this study shows that IgM IgA and IgG are present in purulent and serous maxillary sinus secretion in concentrations that often but not always are comparable to those of serum In purulent secretion IgA is significantly lower and IgG seems to be lower *than in serous secretion* IgA and IgG in sinus secretion are partly serum derived and partly locally produced by the sinus mucosa Furthermore it can be concluded that the maxillary sinus is well suited for the investigation of local immunity, not only because of the accessibility of the secretion and the possibili ty of obtaining repeated specimens, but also because of the possibility of obtaining the etiological microorganism with great certainty

ACKNOWLEDGEMENTS

The authors are deeply indepted to Ass Prof Carl Magnus Eneroth and Dr Renée Norberg for their support and guidance

ZUSAMMANFASSUNG

Fur die vorliegende Arbeit wurde die Verteilung von Im munglobulin im Sekret des Sinus maxillaris bei 17 Patien ten mit Sinusitis maxillaris untersucht Nachweisbare Mengen von IgM fanden sich im Sekret von 15 Patienten wahrend dies hinsichtlich IgA und IgG im Sekret aller Pa tienten der Fall war In eitrigem Sinussekret war der Gehalt an Immunglobulin niedriger als in serosem Sekret und der IgA-Gehalt war erheblich niedriger Von dem Gesamtgehalt an IgA im Sinussekret waren durchschnitt lich 30 Prozent (bei eitrigem Sekret) und 43 Prozent (bei serosem Sekret) örtlich erzeugt Fur IgG lag die ent sprechende Zahl bei 15–18 Prozent wahrend IgM mit dem angewandten Verfahren nicht geschatzt werden konnte Die Arbeit kommt zu dem Schluß daß der Sinus maxil laris ein geeignetes Organ fur die Untersuchung örtlicher Immunitat hinsichtlich bakterieller Infektionen ist

REFERENCES

Bernstein J M Tomasi T B Jr & Ogra P 1974 The immunochemistry of middle ear effusions *Arch Otolaryngol 99* 320

Biberfield G & Sterner G 1971 Antibodies in bronchial secretions following natural infection with Muco plasma pneumoniae *Acta Pathol M crobiol Scand (B) 79* 599

Bing J E & Plum P 1937 Serum proteins in leucopenia *Acta Med Scand 92* 415

Bjorkwall T 1950 Bacteriological examinations in maxil lary sinusitis *Acta Otolaryngol* (Stockh) Suppl *83* 33

Brandtzaeg P 1974 Presence of J chain in human im munocytes containing various immunoglobulin classes *Nature 252* 418

Brandtzaeg P Fjellanger I & Gjeruldsen S T 1967 Localization of immunoglobulins in human nasal mu cosa *Immunochemistry 4* 57

— 1968 Absorption of immunoglobulin A onto oral bac teria in vivo *J Bacteriol 96* 242

Van de Calseyde P Blatton V & Ampe W 1971 T protein pattern of m d lle ear effusion behind drum *Acta Otolaryngol* (Stockh) 71 153

Carenfelt C Eneroth C M Lundberg C & Wretlind B 1975 Evaluation of the antibiotic effect in treatment of maxillary sinusitis *Scand J Infect Dis 7* 259

Carenfelt C & Lundberg C The oxygen tension in maxillary sinus secretion To be published

Deuschl H & Johansson S G O 1974 Immunoglobulins in tracheobronchial secretion with special reference to IgE *Clin Exp Immunol 16* 401

Dixon W J & Massey F J 1957 *Introduction to Statistical Analyses* pp 280 and 289 McGraw Hill Book Company Inc New York

Donovan R Johansson S G O Bennich H & Soothill J F 1970 Immunoglobulins in nasal polyp fluid *Int Arch Allergy Appl Immunol 37* 154

Falk G A Okinaka A J & Siskind G W 1972 Immunoglobulins in the bronchial washings of patients with chronic obstructive pulmonary disease *Am Rev Resp Dis 105* 14

Hanson L Å 1961 Comparative immunological studies of the immunoglobulins of human milk and blood serum *Int Arch Allergy Appl Immunol 18* 241

Heremans J F Crabbe P A & Masson P L 1966 Biological significance of exocrine gamma A immunoglobulin *Acta Med Scand* Suppl *179* 84

Keimowitz R I 1964 Immunoglobulins in normal human tracheobronchial washings A qualitative and quantitative study *J Lab Clin Med 63* 54

Lorin M I Gaerlan P F & Mandel I D 1972 Quantitative composition of nasal secretions in normal subjects *J Lab Clin Med 80* 275

Lonsmann Poulsen H 1974 Interstitial fluid concentrations of albumin and immunoglobulin G in normal men *Scand J Clin Lab Invest 34* 119

Mancini G Carbonara A O & Heremans J F 1965 Immunochemical quantitation of antigens by single radial immunodiffusion *Immunochemistry 2* 235

Mogi G & Honjo S 1972 Middle ear effusions Analysis of protein components *Ann Otol Rhinol Laryngol 81* 99

Mogi G Honjo S Maeda S & Yoshida T 1973 Secretory immunoglobulin A (SIgA) in middle ear effusions *Ann Otol Rhinol Laryngol 82* 30?

Parving H H Nielsen S L & Lassen N A 1974 Increased transcapillary escape rate of albumin IgG and IgM during angiotensin II induced hypertension in man *Scand J Clin Lab Invest 34* 111

Rantanen T & Arvilommi H 1973 Double-blind trial of doxycycline in acute maxillary sinusitis A clinical and bacteriological study *Acta Otolaryngol* (Stockh) 7? 58

Remington J S Vosti K L Lietze A & Zimmerman A L 1964 Serum proteins and antibody activity in human nasal secretion *J Clin Invest 43* 1613

Tomasi T B Jr & Ziegelbaum S D 1963 The selective occurrence of γ A globulins in certain body fluids *J Clin Invest 42* 1552

Tomasi T B Jr & Bienenstock J 1968 Secretory immunoglobulins *Adv Immunol 9* 2

Walsh T E & Cannon P R 1938 Immunization of the respiratory tract A comparative study of the antibody content of the respiratory and other tissues following active passive and regional immunization *J Immunol 35* 31

C Carenfelt M D
Dept of Otolaryngology
Karolinska Sjukhuset
S 104 01 Stockholm 60
Sweden

Acta Otolaryngol 82 131–142, 1976

ZUM POSTNATALEN WACHSTUM DER NASENSCHEIDEWAND

H -J Schultz-Coulon und L Eckermeier

Aus der Hals Nasen-Ohrenklinik der Medizinischen Hochschule Hannover Hannover Deutschland

(Eingegangen am 20 August 1975)

Abstrakt Uber die Anatomie und Histologie und insbesondere uber die Lokalisation und Entwicklung von Wachstumszonen der kindlichen Nasenscheidewand ist verhaltnismäßig wenig bekannt Bei 18 Kindern im Alter von 0 bis 10 Jahren wurden postmortal die Septen in toto entnommen und nach photographischer Dokumentation histologisch und mikroradiographisch untersucht Während zur Zeit der Geburt die noch vollständig knorpelige Septumlamelle mit der Vomerrinne nur durch eine sehr lockere Bindegewebsschicht verbunden ist treten mit zunehmendem Hohenwachstum des Vomers Verknocherungszonen an der dorsalen Vomer-Knorpelgrenze auf Regelmaßig ist beim Neugeborenen septum eine enchondrale Ossifikationszone am Rostrum sphenoidale nachzuweisen die jedoch nicht als Ausgang der Lamina perpendicularis anzusehen ist da sie im Laufe des ersten Lebensjahres verschwindet Die Ossifikation der Lamina perpendicularis beginnt mit dem Auftreten von Ossifikationszentren die zur Zeit der Geburt regelmaßig im dorso-kranialen Abschnitt des knorpeligen Septums gefunden werden Ausdruck des relativ schnelleren Wachstums der Lamina perpendicularis ist die auch im 10 Lebensjahr noch erkennbare enchondrale Ossifikationszone an ihrer Knorpel-Knochengrenze Ebenso läßt sich aus einem Vergleich der anatomischen Beziehungen zwischen knochernen und knorpeligen Septumanteilen in den verschiedenen Altersstufen das gegenuber Vomer und knorpeligem Septum schnellere Wachstum der Lamina perpendicularis entnehmen Dabei finden die starksten Strukturveranderungen innerhalb der ersten sechs Lebensjahre statt anschließend vergroßert sich die Gesamtflache des Sep- [illegible] der enchondra [illegible]

ren Nase führen konnen Bei chirurgischen [illegible] am kindlichen Septum sollte daher die Wachstumszone zwischen Lamina perpendicularis und Knorpel möglichst geschont werden

Wahrend wir uber die embryonale Entwicklung der menschlichen Nasenscheidewand vor allem durch die Arbeiten von Peter (1913, 1938), Richter (1932) und Hillenbrand (1933) eingehende Kenntnisse besitzen, liegen bezüglich des postnatalen Wachstums nur verhältnismaßig sparliche Informationen vor, die zum Teil ebenfalls auf die Mitteilungen der genannten Autoren, hauptsächlich aber auf die systematischen Untersuchungen von Scott (1953, 1954) zuruckgehen Danach ist lediglich bekannt, daß sich im Laufe des ersten Lebensjahres im dorso-kranialen Abschnitt des zur Zeit der Geburt noch vollständig knorpeligen Septums multiple Ossifikationszentren bilden Hillenbrand (1933) beschreibt Ossifikationszentren bereits im 8 Embryonalmo-



Abb 1 Anatomisches Praparat eines Neuge septums Das Mukoperichondrium ist beide fernt V=Vomer R S =Rostrum sphen Crista galli

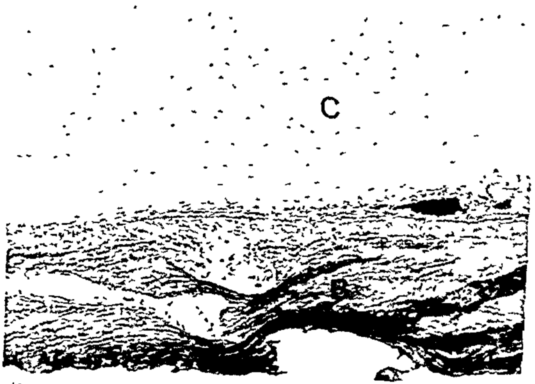


Abb. 2. Longitudinalschnitt durch die Knorpel-Knochengrenze zwischen Vomer und Septumlamelle bei einem Neugeborenenseptum. Der Knorpel (*C*) und Vomerknochen (*B*) sind durch eine lockere Bindegewebsschicht voneinander getrennt. Vergr. 1:125

nat, von denen ausgehend die Lamina perpendicularis durch enchondrale Verknöcherung langsam in ventro-kaudaler Richtung wächst und sich im vierten bis sechsten Lebensjahr mit dem ebenfalls erst postnatal in Höhe wachsenden Vomer knöchern verbindet. Man hat dabei zwei Wachstumsperioden unterschieden. An eine Zeit relativ schnellen Wachstums schließt sich nach dem 7. Lebensjahr eine zweite Periode an, in der sich bis zum Beginn der Pubertät die Größenverhältnisse der Nase nur wenig ändern.

Da in den letzten zwei Jahrzehnten die chirurgischen Eingriffe an der kindlichen Nase und besonders an der kindlichen Nasenscheidewand durch die Publikationen zahlreicher Autoren (Cottle, 1951; Jennes, 1964; Masing, 1971; Murr, 1970; Kastenbauer, 1973; Hellmich, 1974; Pirsig, 1974 u. v. a.) zunehmend in den Mittelpunkt des Interesses gerückt wurden, erschien es uns wünschenswert, die Kenntnisse über die anatomischen und histologischen Verhältnisse der wachsenden Nasenscheidewand zu erweitern. Dabei gingen wir insbesondere der Frage nach, ob sich in den verschiedenen Altersstufen bestimmte Wachstumszonen in den knorpeligen und knöchernen Septumanteilen mikroskopisch-anatomisch abgrenzen lassen, denen der Rhinochirurg unter dem Gesichtspunkt einer ungestörten Weiterentwicklung der inneren und äußeren Nase Beachtung schenken sollte.

MATERIAL UND METHODE

Bei 18 Kindern im Alter von 0–10 Jahren (9 Neugeborene, 9 Kinder im Alter von 0,6, 1, 1,6, 4, 7, 8, 10 Jahren) entnahmen wir unmittelbar postmortal die Nasenscheidewand in toto von der vorderen Schädelgrube aus, wobei besonderer Wert auf die Erhaltung der Knorpel-Knochengrenze zwischen knorpeligen Septum und Lamina perpendicularis bzw. Vomer und Rostrum sphenoidale gelegt wurde. Nachdem wir die Septen beiderseits



Abb 3 Knochenbildungsfront an der Knorpel-Knochen grenze zwischen dorsaler Vomeroberkante (*B*) und knorpeligem Septum (*C*) zehnjahriges Septum Die breiten Osteoidsaume (*Pfeile*) zeigen lebhaften Knochen anbau Vergr 1 125

vom Mukoperichondrium befreit hatten, wurden sie photographisch dokumentiert und der besseren Ubersicht wegen halbschematisch nachgezeichnet Anschließend wurden sie in Metacrylat eingebettet in longitudinaler sowie transversaler Richtung geschnitten und nach verschiedenen Methoden (Hamatoxylin-Eosin , Azur Eosin Goldner-Farbung, Toluidinblau) gefarbt

Um die Aktivitat des Knochenanbaus und Knochenumbaus besser abschatzen zu konnen, haben wir die Knorpel-Knochengrenzen, an denen wir im gefarbten Praparat Wachstumsvorgänge erkennen konnten, zusätzlich mikroradiographisch untersucht, eine Methode, die besonders Jowsey (1966) zur Darstellung der Aktivitatsphasen im Knochen empfohlen hat Bei diesem Verfahren wird ein zunachst etwa 100 μ dicker Hartschnitt durch vorsichtiges Schleifen bis zu einer Dicke von nur noch 50 μ verdunnt und anschließend mit einem speziellen Gerat gerontgt Infolgedessen kommen nur mineralisierte Gewebe zur Darstellung, d h also nur Knochen mit Knochenanbau- bzw -abbauzonen, nicht aber Knorpel oder Bindegewebe

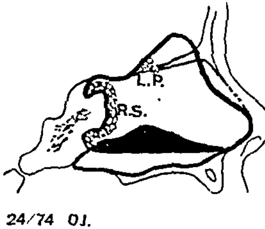


Abb 4 Anatomie eines Neugeborenenseptums (halbschematisch) Die enchondrale Ossifikationszone Rostrum sphenoidale (*R S*) sowie die Ossifika zentren der Lamina perpendicularis (*L P*) sind Schraffur gekennzeichnet Maßstab ca 1 1,5

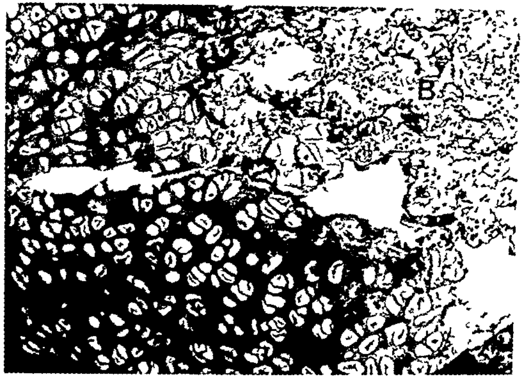


Abb 5 Enchondrale Ossifikationszone am Rostrum sphenoidale bei einem Neugeborenenseptum *C*=Knorpel *B*=neugebildeter Knochen Vergr 1 125

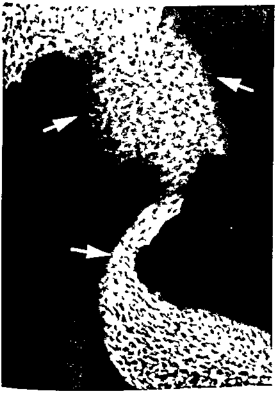
ERGEBNISSE

as zweiteilige Präparat eines Neugeborenseptums ist in Abb 1 dargestellt Einschließlich der Crista galli besteht es aus einer zigen Knorpellamelle die basal in die nahezu horizontal verlaufende Vomerrinne hineingestellt ist Die Verbindung zwischen Knorpel und Vomer bzw zwischen Knorpel und Pramaxilla ist noch so locker daß sich nach Ablosen des Mukoperichondriums der knorpelige Septumanteil fast muhelos aus der Vomerrinne herauslosen laßt Histologisch (Abb 2) stellt sich diese Verbindung als schmale Bindegewebsschicht mit vorwiegend longitudinal verlaufender Faserrichtung dar die zur einen Seite als Perichondrium in typischer Weise kontinuierlich in das Knorpelgewebe ubergeht sich aber auch nach der anderen Seite ohne scharfe Grenzen als Periost mit dem Vomer vereinigt Dieser lockere Knorpel–Knochenkontakt macht es vorstellbar daß bei Neugeborenen und Sauglingen schon verhaltnismaßig geringfugige Nasentraumen zur Subluxation des vorderen Septumabschnitts fuhren können wie es von Kirchner (1955) Steiner (1959) Jeppesen & Windfeld (1972) und anderen Autoren mehrfach geschildert wurde

Im ventralen Septumabschnitt bleiben auch bei alteren Kindern Vomer und Knorpellamelle nur bindegewebig miteinander verbunden jedoch wird diese Verbindung durch die Entwicklung straffer kollagener Faserzuge (besonders im Bereich der Spina nasalis anterior und der Pramaxilla) mit fortschreitendem Alter immer fester Dagegen treten im dorsalen Septum mit zunehmendem Höhenwachstum des Vomers zahlreiche Umbauzonen an der Kontaktstelle zwischen Vomeroberkante und knorpeligem Septum auf die Knorpelabbau und gleichzeitigen Knochenanbau im Sinne einer Knochenbildungsfront mit appositioneller Ossifikation erkennen lassen Diese Umbauzonen finden sich stel



Abb 6 Knochenbildungsfront an der Knochen–Knorpelgrenze zwischen Rostrum sphenoidale und knorpeligem Septum bei einer einjahrigen Nasenscheidewand Der histologische Schnitt (Abb *6a*) zeigt die von Bindegewebe eingeschlossenen Chondrozyteninseln (*C*) als Ausdruck allmahlichen Knorpelabbaus Das appositionelle Knochenwachstum ist an den Osteoidsaumen (*Pfeile*) zu erkennen *B*=Knochen Vergr 1 100 Auf der mikroradiographischen Aufnahme (Abb *6b*) drucken die zahlreichen schwacher mineralisierten Zonen (*Pfeile*) einen lebhaften Knochenanbau aus Vergr 1 125



lenweise bereits beim einjahrigen Septum und sind bei alteren Septen regelmäßig am gleichen Ort nachzuweisen Ein entsprechender Abschnitt aus der Vomer–Knorpelgrenze des zehnjährigen Septums, das wir untersuchen konnten, ist in Abb 3 wiedergegeben kleine, von Bindegewebe umschlossene Knorpel inseln liegen in unmittelbarer Nahe des Knochens, dessen breite Osteoidsäume von lebhaftem Knochenanbau Auskunft geben

Ahnlich wie bei der Lamina perpendicularis beruht also auch beim Vomer die Großenzunahme seiner dorsalen Flache teilweise auf einer allmahlichen Verknocherung der dorsalen knorpeligen Septumlamelle mit dem allerdings wesentlichen Unterschied, daß hier keine enchondrale Ossifikation vorliegt (wie bei der Lamina perpendicularis), sondern daß ein langsames appositionelles Knochenwachstum dem vorausgehenden Knorpelabbau folgt

Dagegen erscheint die Kontakt zwischen Knorpellamelle und Keilbein



Abb. 7. Enchondrale Verknöcherungszone an der Knorpel–Knochengrenze der Lamina perpendicularis bei einer achtjährigen Nasenscheidewand *C*=Knorpel, *B*=Knochen, Vergr. 1:125

(Rostrum sphenoidale) beim Neugeborenen regelmäßig als typischer enchondraler Ossifikationsherd (Abb. 4, 5): Die Knorpelzellen [illegible] sich zum sog. Säulenknorpel auf, wer-[illegible] blasig aufgetrieben und von Chondro-[illegible]sten abgebaut; unmittelbar hinter der Knorpelabbauzone treten die ersten Knochenbälkchen auf. Diese sphenoidale Ossifikationszone ist nicht als Ausgangspunkt der Lamina perpendicularis zu betrachten, da die ausschließlich enchondrale Verknocherung sehr bald nach der Geburt gestoppt wird, so daß sich schon beim einjähriger Septum ein ganz anderes Bild an dieser Stelle ergibt (Abb. 6*a*): Auch jetzt laßt sich noch Knochenanbau durch die Anwesenheit von Osteoidsäumen und breiten, schwächer mineralisierter Randzonen (Abb. 6*b*) nachweisen, doch hat die Wachstumsgeschwindigkeit in diesem Bereich offensichtlich erheblich abgenommen. Nach Verschwinden der enchondralen Ossifikation beobachtet man hier also die gleichen Wachstums- und Verknöcherungsvorgänge wie zwischen Vomer und Knorpel. Indem sie langsam fortschreiten und spater mit der vorwachsenden Lamina perpendicularis verschmelzen, führen sie allmählich zur vollständigen Verknöcherung der dorsalen Septumhälfte.

Ossifikationszentren der Lamina perpendicularis konnten wir unmittelbar hinter der Crista galli am kranialen Septumrand (schematisch in Abb. 4) regelmaßig bei Septen von Neugeborenen in Form von an mehreren Stellen in den Knorpel eingesproßten Bindegewebssträngen mit eingestreuten winzigen Knochenbälkchen nachweisen. An der Knorpel–Knochengrenze der Lamina perpendicularis bleibt eine, wenn auch sehr schmale, so doch ausschließlich enchondrale Ossifikationszone bis mindestens zum 10. Lebensjahr erhalten, die man wohl als Ausdruck eines relativ schnelleren Wachstums in diesem Bereich interpretieren muß. Der histologische Schnitt (Abb. 7), der von einem achtjährigen Septum stammt, zeigt die bekannte Säulen-

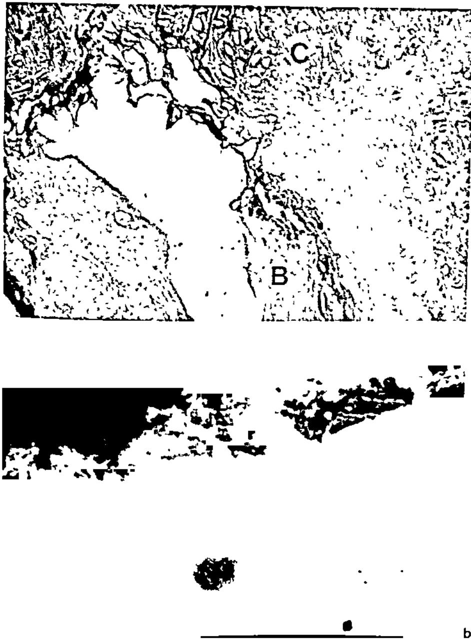
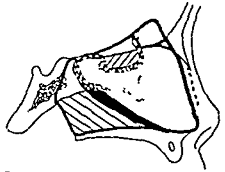


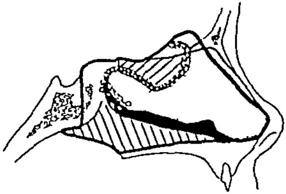
Abb. 8 … an der Knor-

Vergr. 1 100 Die mikroradiographische Aufname der gleichen Ossifikationszone (Abb 8*b*) zeigt das für eine enchondrale Verknöcherung typische „mottenfraßähnliche“ Bild mit zahlreichen schwächer mineralisierten Zonen Vergr 1 125.

formation der Knorpelzellen zwar nicht mehr so deutlich, beweist jedoch durch die unmittelbare Nachbarschaft der aufgetriebenen und sich langsam auflosenden Knorpelzellen zu dem im Aufbau befindlichen Knochengewebe, daß es sich um eine rein enchondrale Verknöcherung handelt. Beim zehnjährigen Septum laßt sich ein ganz ähnliches Bild beobachten (Abb 8*a*), jedoch ist als Ausdruck eines langsameren Wachstums die Ver-



3/74 0.6 J



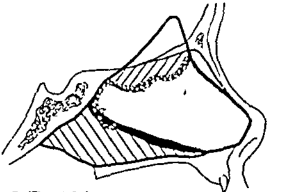
18/74 1 J.

Abb 9 Anatomie einer 0 6jahrigen Nasenscheidewand (*oben*) und einer 1 0jahrigen Nasenscheidewand (*unten*) Halbschematisch Maßstab ca 1 1 1

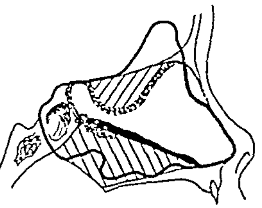
ngszone noch schmaler geworden und die ssifikationsvorgange sind stellenweise unterbrochen Die mikroradiographische Aufnahme (Abb 8*b*) des gleichen Praparates zeigt zahlreiche schwacher mineralisierte Zonen als Zeichen eines aktiven Knochenanbaus

Auch der makroskopisch anatomische Vergleich der Septen in den verschiedenen Altersstufen läßt das gegenuber Vomer und knorpeligem Septum schnellere Wachstum der Lamina perpendicularis wahrend der ersten 5–6 Lebensjahre erkennen Bei einer halbjahrigen Nasenscheidewand (Abb 9) fanden wir die Lamina perpendicularis als kleinen verknocherten Bezirk am oberen Septumrand dorsal von der Crista galli Nach dem ersten Lebensjahr hat sie sich uberwiegend in dorsaler und ventraler Richtung entwickelt hat die Crista galli jedoch noch nicht vollstandig in die Verknocherung einbezogen Erst wenn etwa in der Mitte des zweiten Lebensjahres auch die Crista galli vollstandig ossifiziert ist (Abb 10) setzt ein vorzugsweise nach kaudal gerichtetes Wachstum der Lamina perpendicularis ein jedoch hat sie auch nach dem vierten Lebensjahr den in die Höhe gewachsenen Vomerrand noch nicht erreicht. Im 6 Lebensjahr (Abb 11) ist der knocherne Kontakt mit dem Vomerrand und dem Rostrum sphenoidale bereits hergestellt danach andern sich die anatomischen Beziehungen zwischen knorpeligen und knochernen Septumanteilen nur noch geringfügig wenngleich die Gesamtflache des Septums vom sechsten bis zum zehnten Lebensjahr deutlich zugenommen hat

Eine ungefahre Vorstellung von der alters



2/74 16 J



1/74 4 J

Abb 10 Anatomie einer 1,6jährigen Nasenscheidewand (*oben*) und einer 4jährigen Nasenscheidewand (*unten*) Halbschematisch Maßstab ca 1 1 1

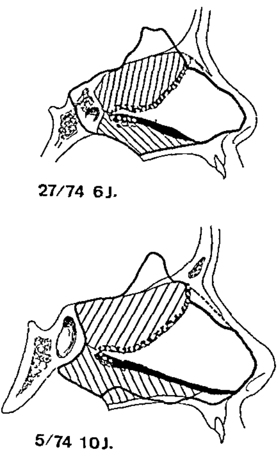


Abb 11 Anatomie einer 6jährigen Nasenscheidewand (*oben*) und einer 10jährigen Nasenscheidewand (*unten*) Halbschematisch Maßstab ca 1 1 4

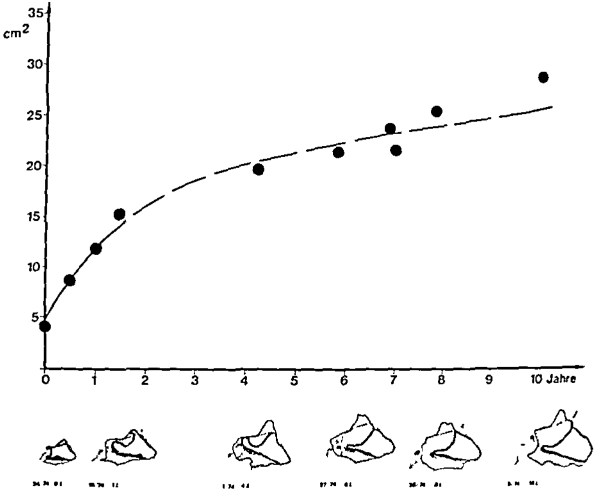
abhängig sich ändernden Wachstumsgeschwindigkeit der gesamten Nasenscheidewand kann man sich verschaffen wenn man die Produkte aus Länge und Höhe der einzelnen Septen als relatives Flächenmaß in ein Koordinatenkreuz einträgt (Abb 12) Nachdem sich in der Mitte des ersten Lebensjahres die Septumfläche bereits nahezu verdoppelt hat, sinkt die Wachstumsgeschwindigkeit mit zunehmendem Alter laufend ab Das entspricht den Untersuchungsergebnissen von Scott (1953) insofern als das Septum in den ersten fünf bis sechs Lebensjahren schneller wächst als nach dem sechsten Lebensjahr jedoch erscheint angesichts unserer geschätzten Wachstumskurve eine genaue zeitliche Abgrenzung von zwei Perioden mit unterschiedlich schnellem Wachstum – wie Scott es getan hat – kaum möglich, da sich die Wachstumsgeschwindigkeit nicht plötzlich zu einem bestimmten Zeitpunkt, sondern ganz allmählich ändert

Hinzugefügt sei daß es erhebliche individuelle Unterschiede in der Anatomie der kindlichen Nasenscheidewand gibt Während bei unserem sechsjährigen Septum die Lamina perpendicularis den Vomerrand bereits erreicht hatte waren bei einem siebenjährigen Septum die beiden knöchernen Septumanteile noch durch einen breiten Knorpelstreifen getrennt Offensichtlich kann also die Verschmelzung von Vomer und Lamina perpendicularis erst sehr viel später erfolgen So hat Morrison (1970) ebenfalls darauf hingewiesen, daß man auch jenseits des zehnten Lebensjahres noch Septen finden kann, bei denen der knorpelige Teil bis zum Keilbeinkörper reicht

DISKUSSION

Zusammenfassend darf man zunächst feststellen, daß das postnatale Wachstum der Nasenscheidewand in erster Linie durch die schnelle Vergrößerung der knöchernen Septumanteile – insbesondere der Lamina perpendicularis – charakterisiert wird, und daß diese Entwicklungsvorgänge sich hauptsächlich in den ersten fünf bis sechs Lebensjahren abspielen Regelmäßig lassen sich schmale Wachstumszonen an der Knorpel-Knochengrenze der Lamina perpendicularis, des Rostrum sphenoidale und der dorsalen Vomeroberkante nachweisen, wobei das lebhafteste Wachstum am Übergang zwischen Lamina perpendicularis und knorpeligem Septum stattzufinden scheint Innerhalb der knorpeligen und knöchernen Septumabschnitte sind irgendwelche Wachstumszonen morphologisch jedoch nicht abgrenzbar

Die Tatsache des relativ schnellen Wachstums der Lamina perpendicularis könnte durchaus die Vermutung von Pirsig (19 stützen, daß im ethmoidalen Septumkn



bb 12 Postnatale Wachstumsgeschwindigkeit der kind hen Nasenscheidewand (Ordinate Produkt aus Lange Höhe der Septen in cm² Abszisse Lebensalter)

ein wichtiges, für das Höhenwachstum der Nase entscheidendes Wachstumszentrum liegt Weitere Anhaltspunkte für diese Hypothese haben wir aus unseren morphologischen Studien allerdings nicht entnehmen können Manche amerikanische Autoren (Morrison, 1970, Stevens, 1970) legen das primare Wachstumszentrum an die Verbindungsstelle zwischen knorpeligem Septum und Pramaxilla, d h in einen Bereich, der etwa dem Loc Kiesselbachii entspricht und der im amerikanischen Schrifttum nach Mosher (1909, zit n Morrison) als "the area of the septum mosaic" bekannt ist Diese Ansicht gründet sich auf die Ergebnisse von Wexler & Sarnat (1961), die bei jungen Kaninchen Knorpelresektionen (einschließlich Mukoperichondrium) aus der entsprechenden Septumregion vornahmen und daraufhin Storungen der Mittelgesichtsentwicklung beobachteten Diesbezuglich sind auch die Untersuchungen von Long et al (1968) bemerkenswert, die ebenfalls bei Kaninchen auf autoradiographischem Wege eine verstärkte Proliferationsaktivität im antero-inferioren Septumbereich sowie an der enchondralen Wachstumszone der septoethmoidalen Verbindungsstelle nachweisen konnten

Man wird es künftigen Untersuchungen uberlassen müssen, ob tatsächlich innerhalb des knorpeligen Septums bestimmte Wachstumszentren lokalisiert werden können Im

merhin aber darf man aus der Ähnlichkeit der enchondralen Ossifikationszone zwischen Lamina perpendicularis und Septumknorpel mit einer Epiphysenfuge der langen Röhrenknochen schließen, daß dem ungestörten Wachstum des Knorpels die entscheidende Rolle für die regelrechte Entwicklung der äußeren und inneren Nase zukommt Hierfür sprechen die bereits erwähnten Untersuchungen von Wexler & Sarnat, die Befunde von Pétrović et al (1969), die die unabhängige Wachstumspotenz des Septumknorpels von Ratten in vitro zeigen konnten, und hierfür sprechen vor allem die zahlreichen klinischen Beobachtungen von Wachstumsstörungen der äußeren Nase bei Fällen mit kongenitaler Aplasie des knorpeligen Septums oder nach frühkindlichen Septumtraumen oder Septumabszessen Wenn Hayton (1918) nach submuköser Septumresektion bei 31 Kindern in 33% der Fälle eine Verbreiterung des Nasenrückens und in 20% ein angedeutetes Einsinken der Nase sah, und wenn Ombredanne (1942) ebenfalls nach submuköser Septumresektion bei 50 Kindern in sogar 60% der Fälle eine Abflachung der knorpeligen Nase mit weicher Columella feststellen mußte so erscheint es nicht abwegig, wenn man diese Spätdeformierungen der äußeren Nase auf das gestörte Knorpelwachstum infolge der durchgeführten Resektionen und insbesondere auf eine Zerstörung der enchondralen Ossifikationszone zurückführt

Damit können wir aus unseren histologisch anatomischen Untersuchungen im Hinblick auf die Septumchirurgie im Kindesalter die gleichen Richtlinien ableiten wie sie seit Cottle (1939, 1951) von vielen Autoren (Masing, 1971 Kastenbauer 1973 Pirsig & Knahl, 1974 u a) vertreten werden Eine submuköse Septumresektion nach Killian verbietet sich auf Grund der damit unvermeidbar verbundenen groben Zerstörung von wachsenden Strukturen – ganz abgesehen davon, daß diese Methode den gerade bei Kindern besonders häufig deformierten oder subluxierten vorderen Septumabschnitt nur mangelhaft korrigieren kann Jede Septumplastik im Kindesalter sollte so konservativ d h so strukturerhaltend wie möglich durchgeführt werden sollte sich nur auf die Reposition des fehlgestellten Septums beschränken und sollte mit Knorpel- oder Knochenresektionen vor allem in den vorderen und mittleren Septumabschnitten äußerst zurückhaltend sein Darüber hinaus sind wir der Ansicht, daß man in den ersten sechs Lebensjahren angesichts der steiler verlaufenden Wachstumskurve mit der Indikation zu rhinoplastischen Maßnahmen besonders vorsichtig umzugehen hat

SUMMARY

Up to now little is known about the anatomy and histology and especially about the germinative zones of the growing nasal septum We totally removed 18 septa of children between 0 and 10 years immediately after death After photographical documentation these septa were studied histologically and microradiographically It was found that at birth the connection between the cartilaginous septum and vomer only consists of a small and very loose layer of connective tissues During the first year of life several ossification areas appear between vomer and cartilage in the dorsal part of the septum An enchondral ossification zone at the rostrum sphenoidale was regularly seen in the neonatal septum but disappears soon after birth The development of the perpendicular plate starts from multiple ossification centers regularly to be found in the dorso-cranial part of the neonatal septum The perpendicular plate is the fastest growing part of the septum and even in the tenth year of life still shows an enchondral ossification zone around its border The shape and the size of the septum changes most during the first six years subsequently septal growth slows down markedly As the enchondral ossification zone resembles an epiphysis cartilage it is assumed that lesions in this area may lead to growth disturbances of the inner and outer nose It is therefore emphasized that regarding rhinoplasty in children this ossification zone should be preserved by the surgeon

LITERATUR

Cottle M H 1939 Nasal septum surgery in children *Rev Jll Med J* 161

— 1951 Nasal surgery in children *Eye Ear Nose Throat* *30* 32

Hayton Ch H 1918 An investigation into the results of the submucous resection of the septum in children *Int Cbl Laryngol Rhinol 34* 285

Hellmich S 1974 Die Therapie des frischen Septumabszesses *HNO* (Berl) *22* 278

Hillenbrand K 1933 Entwicklung Bau und Formveranderungen der menschlichen Nasenscheidewand im fetalen Leben *Arch Ohren Nasen Kehlkopfheilkd 135* 1
Jennes M L 1964 Corrective nasal surgery in children *Arch Otolaryngol 79* 145
Jeppesen F & Windfeld J 1972 Dislocation of the nasal septal cartilage in the newborn *Acta Obstet Gynecol Scand 51* 5
Jowsey J 1966 Quantitative microradiography A new approach in the evaluation of metabolic bone disease *Am J Med 40* 485
Kastenbauer E 1973 Außere Nase und Nasenscheidewand *Z Laryngol Rhinol 52* 597
Kirchner J A 1955 Traumatic nasal deformity in the newborn *Arch Otolaryngol 62* 139
Long R Greulich R C & Sarnat B G 1968 Regional variations in chondrocyte proliferation in the cartilaginous nasal septum of the growing rabbit *J Dent Res 47* 505
Masing H Eingriffe an der Nase Die Chirurgie der außeren Nase und der Nasenscheidewand In *Kurze HNO Operationslehre* (hrsg von G Theissing) Verlag G Thieme Stuttgart 1971
Morrison L E 1970 Some relationships between embryology and surgery of the nose *Int Rhinology 8* 185
Mosher H B 1970 zit n L E Morrison
Murr G 1970 Resektion der Nasenscheidewand im Kindesalter *Acta Otolaryngol* (Stockh) *69* 371
Ombrédanne M 1942 Les déviations traumatiques de la cloison chez l enfant avec obstruction nasale Traitement chirurgical et resultats éloignés *Arch Fr Pediatr 1* 20
Peter K *Atlas der Entwicklung der Nase und des Gaumens beim Menschen* Verlag G Fischer Jena 1913
— Die Nase des Kindes In *Handbuch der Anatomie des Kindes* (hrsg von K Peter G Wetzel F Heiderich) Bd II Verlag J Bergmann München 1938
Pétrović A Charlier J P & Herrmann J 1969 Les mécanismes de croissance du crane Recherches sur le cartilage de la cloison nasale et sur les sutures craniennes et faciales de jeunes rats en culture d'organes *Bull Ass Anat 143* 1376
Pirsig W 1973 Das akute Nasentrauma beim Kind *Z Laryngol Rhinol 52* 265
— 1974 Die deformierte Neugeborenennase *HNO* (Berl) *22* 1
Pirsig W & Knahl R 1974 Rhinoplastische Operationen bei Kindern Erfahrungen an 92 Fällen *Z Laryngol Rhinol 53* 250
Richter H 1932 Die normale Entwicklung der menschlichen Nase in Sonderheit der Siebbeinzellen *Arch Ohren Nasen Kehlkopfheilkd 131* 265
Scott J H 1953 The cartilage of the nasal septum *Br Dent J 95* 37
— 1954 The growth of the human face *Proc R Soc Med 47* 91
Steiner A 1959 Certain aspects of nasal trauma in the prenatal–natal period *Md State Med J 8* 557
Stevens S L 1970 Genesis of septal deformities *Int Rhinology 8* 165
Wexler M R & Sarnat B G 1961 Rabbit snout growth *Arch Otolaryngol 74* 305

Dr med H -J Schultz Coulon
HNO Klinik der Med Hochschule
Postfach 610180
D-3000 Hannover 61
Deutschland

Acta Otolaryngol 82 143–146 1976

CERVICAL THYMIC CYSTS

N J Johnsen and P Bretlau

From the University ENT Department Rigshospitalet Copenhagen Denmark

(Received October 9 1975)

Abstract A review of cervical thymus cysts and a case report are presented This ben gn lesion was as in most cases previously reported situated laterally on the neck deep to the anterior border of the sternocleidomastoid muscle and thus s mulating a common branch al cyst pre operatively But unlike the latter most cases of cervical thymus cysts are recognised during childhood They are bel eved to develop in remnants of thymic tissue which have failed to descend from the third branch al pouch into the mediastinum in the 6th to 8th week of embryonic life

The congenital thymic cyst is a rare tumour which can be situated in the neck or in the mediastinum (Sørensen et al 1956 Le Roux 1962 Oldham & Sabiston 1967) In the fol lowing report only cysts in the neck are con cerned Polloson & Piery in 1901 were the first to attempt a partially excision of a cervi cal thymic cyst In 1944 Hyde et al reported the first successful removal of an aberrant cystic Thymus from the neck of a 5 year old boy Most cases of cervical thymic cysts have been published in recent years and they ap pear to constitute a distinct entity In 1974 Fahmy made a thorough review of 31 pre viously operated cases and stated their common clinical and pathological pattern About two thirds occur in the first decade of life and most of the remainder in the second In contrast to this the majority of the common branchial cyst occur after the second decade Males are affected twice as often as females The cysts seem to be more common on the left side and are usually situated deep to the anterior border of sternocleidomastoid muscle adjacent to the carotid sheet About half of the cases have solid extensions down into the mediastinum The majority of the cysts are multilocular and contain an opaque yellow brown fluid filled with cholesterolcrystals Microscopically the cyst wall consists of reg ularly arranged thymus tissue with Hassalls corpuscles which in some cases show cystic degeneration Areas with cholesterol crystals surrounded by foreign body giant cells are often found The epithelium lining the cysts ranges from a single layer of flattened squa mous or low cubodial cells to a multilayered stratified squamous epithelium

The purpose of this paper is to present an additional case of cervical thymic cyst, which has most of the characteristic features and to discuss the pathogenesis of these cysts

Case Report

In an otherwise healthy 7 year-old boy a pain less swelling in the left side of the neck was observed following an upper respiratory tract infection 2 weeks before admission to hospi tal

Examination revealed a soft indolent prob- ably cystic mass the size of a mandarin on the left side of the neck along the lower an terior border of the sternocleidomastoid mus cle The mass was not pulsating and did not move with swallowing On forced expiration it increased in size There was no redness

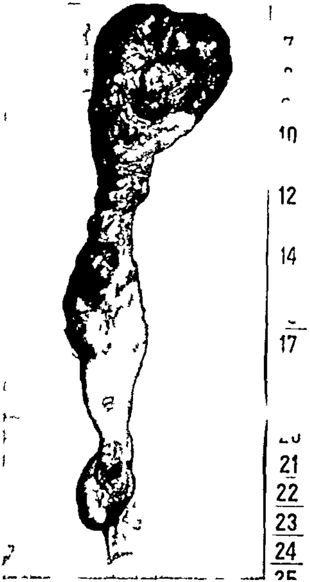


Fig 1 Gross appearance of the lobulated club-formed tumour

induration over the tumour and no associated lymphadenopathy could be palpated An X ray of the chest and routine laboratory tests including a complete blood cell count revealed nothing abnormal A common branchial cleft cyst was the most likely preoperative diagnosis and surgical exploration was carried out through a transverse incision on the left side of the neck The tumour an encapsulated cystic mass was exposed and found to lie superficially to the carotid sheath beneath the anterior edge of the sternocleidoid muscle The lower portion of the mass extended downward into the superior mediastinum as a solid cord and had to be cut before the cyst could be removed The postoperative course was uneventful

Pathology

Gross examination (Fig 1) revealed a yellow bluish lobulated club formed tumour measuring 13 cm in length and 4 cm in diameter at the upper pole and 2 cm at the lower On section the specimen was multilocular with one large and several smaller cysts filled with a dark brown fluid The lining of the cyst walls was smooth The walls consisted of firm white tissue and varied from 1 to 5 mm in thickness

Microscopy (Fig 2)

The large cyst was lined with flattened 2-3 layered squamous epithelium In the wall of this lumen many small cysts lined with the same type of epithelium were found Among the cysts there were abundant amounts of thymic tissue with formation of cortical and medullary areas containing Hassall s corpuscles which in some cases showed cystic degeneration Numerous areas with cholesterol clefts surrounded by histiocytes and foreign body giant cells and areas with recent blood clots mixed with inflammatory cells were also seen

DISCUSSION

Embryologically thymus is a paired organ derived from the third pharyngeal pouches (Hamilton et al 1972) During the sixth week of fetal life the thymic primordia develop as a ventral sacculation on each side (Fig 3) By continued growth these sacculations elongate caudally as tubular structures which soon become solid due to epithelial proliferation The connection with the third pharyngeal pouch is severed by the seventh week and the two primordia then fuse in the midline and descend to their final position in the anterior mediastinum attached to the pericardium during the eighth week of embryonic life



Fig 2 The cyst wall lined with squamous epithelium containing abundant amounts of thymic tissue with Hassall's corpuscles Haematoxylin and eosin ×40

Remnants of thymic tissue may be implanted anywhere along the normal route of descent and are occasionally found among tissues removed during exploratory operations for parathyroid adenoma (Ellis, 1967) or at surgery for unrelated thyroid diseases (Indeglia et al., 1967) The pathogenesis of cyst formation within thymic tissue is still a matter of controversy Speer (1938) originally suggested that thymic cysts may develop in a number of different ways However, it seems quite possible either that the cysts arise as

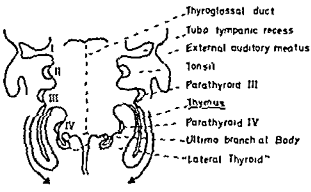


Fig 3 Diagram of the branchial apparatus

result of cystic degeneration in Hassall's corpuscles (Ellis, 1967) or that they are derived from remains of the thymopharyngeal tubules (Fahmy, 1974) Since the thymus reaches its greatest size in the years before puberty it is not surprising that the peak incidence of cervical thymic cysts occurs in childhood

Although a case of malignant epithelioma in solid undescended thymus tissue in the neck of a child have been reported (Pirkey, 1954) no malignant changes in association with a cervical thymic cyst have yet been published Nor have another possible complication myasthenia gravis been recorded It is well known that the thymus plays an important role in the development of immunological competence A thymic cyst in the neck usually represents only a part of the patient's thymic tissue and so far there have been no reports on immunological deficiencies following removal of a cervical thymic cyst

The differential diagnosis of an asymptomatic lateral cystic mass in the neck of a child must include cystic hygroma, common branchial cyst and a possible malignancy The increasing number of cervical thymic cysts published in recent years shows that this type of cysts also should be taken under consideration

ZUSAMMENFASSUNG

Eine Übersicht über cervicale Thymuszysten und ein Fall ...

... deus lokalisiert und simulierte so präoperativ eine gewöhnliche branchiogene Halszyste Aber im Gegensatz zu diesen kommen die cervicalen Thymuscysten am häufigsten bei Kindern vor Man vermutet daß diese Zysten vom Thymusgewebe entwickelt sind das beim Abstieg der Thymusanlage in dem Mediastinum während der 6–8 Embryonalwoche in der dritten Branchialfurche hinterlassen wurde

REFERENCES

Ellis H A 1967 Cervical thymic cysts *Brit J Surg* *54* 17

Fahmy, S 1974 Cervical Thymic Cysts their pathogenesis and relationship to branchial cysts *J Laryng* *Otol 86* 47

Hamilton W J Boyd J D & Mossman H W 1972 *Human Embryology* 4th ed W Heffer & Sons Ltd Cambridge

Hyde T L, Sellers E D & Owen M 1944 Thymic cyst of the neck report of a case *Tex State J Med* *39* 539

Indeglia R A Shea M A & Grage T B 1962 Congenital cysts of the thymus gland *Arch Surg 83* 149

[illegible] ... of the media [illegible]

primitiv du thymus *Providence Med 15* [illegible]

Speer, F D 1938 Thymic cysts report of a thymus presenting cysts of three types *NY M Coll And Flower Hosp Bull 1*, 142

Sorensen H R Jorgensen J B Thomsen G Westergaard Nielsen V & Eskelund V 1956 Thymic tumours and cysts *Acta Chir Scand 110* 393

N J Johnsen M D
University ENT Department
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen
Denmark

Acta Otolaryngol 82 147–150, 1976

A NEW METHOD FOR STRENGTHENING PALATAL CLOSURE DEFECTS OF THE HARD PALATE

A Biorklund Hj Koch and K I Pettersson

From the Department of Otolaryngology University Hospital Lund Sweden

(Received August 21 1975)

Abstract Closure of minor defects in the hard palate can be done with local flaps There is often a risk of perfora t on of the reconstructed area In the case reported this complication was obvious due to a large thin flap-covered area In strengthening the reconstruction fascia lata and Surgicel® was used The postoperative result was excellent and the rehabilitation of the patient was without complications

Reconstruction of the palatal process of the maxillary bone is sometimes a clinical prob lem Major defects can be replaced with prosthesis Minor defects can be closed with local flaps However, after reconstruction with flaps, the area can be very vulnerable and can perforate In this case report a method for the strengthening of such flaps is described

CASE REPORT

An 11 year old boy was admitted with a 1 cm large soft tumour in the left hard palate close to the midline The alveolar process was not affected Fine needle aspiration biopsy dem onstrated a pleomorphic adenoma but a highly differentiated muco-epidermoid carcinoma of low grade malignancy could not be excluded The tumour was extirpated leaving the bone intact At histopathology the tumour was clas sified as a muco-epidermoid carcinoma as in the cytology report However the tumour had been resected with small margins and a new operation was indicated At the second opera tion all soft tissue and bone covering an area from the midline to the maxillary sinus was extirpated The anterior–posterior extension of the operation was 3 cm The periosteum to the nasal cavity was left in a denuded state towards the oral cavity The region had some granular changes but frozen sections did not demonstrate any cancer However, after ex amination of permanent slides, cancer was found A third operation was therefore planned

Operation

The operation field was revised All perioste um and mucosa in the operation area was ex tirpated leaving a defect to the nasal cavity of 1½×4 cm (Fig 1) To reconstruct this surgical defect a vomerin flap was raised and based on the upper left septal wall The flap included the periosteum and mucosa A similar flap was raised from the lateral wall of the nasal cavity beneath the inferior concha A Portex tube size no 4 was placed into the nasal cavity to support the flaps from above The flaps were proximated and sutured with one layer of 3/0 dexon stitches (Fig 2) The rec… was large and very thin The risk … tion was therefore considered

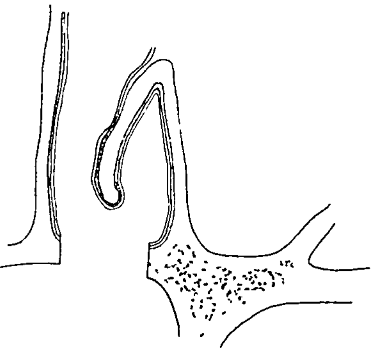


Fig 1 Lower part of left nasal cavity with the defect in the hard palate after surgery of tumour and adjacent tissues

this complication a free graft of fascia lata was placed into the palatal defect and extended in to the nasal cavity beneath the vomerin and lateral flaps The residual defect in the palate was filled with Surgicel® Both the fascia and Surgicel® were held in position by a palatal , fixed to the teeth (Fig 3)

Follow up

The patient was fed via a naso-gastric tube for 14 days No antibiotics were given The palatal plate was changed after 10 days and new plate was made This plate was kept for 4 more weeks

Six months after operation the defect had

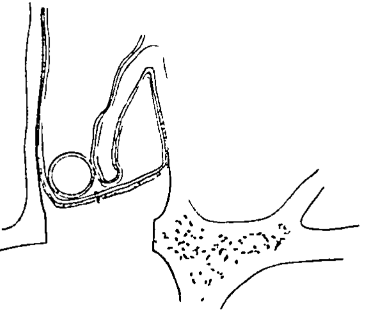


Fig 2 Medial and lateral flaps are raised and sutured beneath the Portex tube

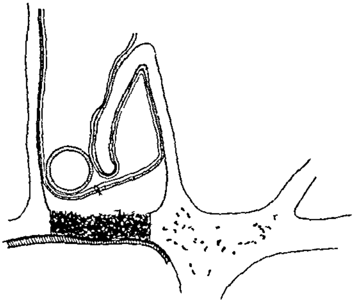


Fig 3 Fascia lata is placed into the nasal cavity The defect in the hard palate is filled with Surgicel® The substitutes are held in position by a palatal plate

healed and the mucous membrane in the hard plate was smooth and thin In the nasal cavity there was a normal passage and no defects were seen The patient has been followed up for 2½ years There is no recurrence and no metastasis

DISCUSSION

Recently Hjertman & Eneroth (1970) have discussed the classification of tumours of the palate In their material of 383 cases salivary gland tumours were more commonly found in the hard palate The prognosis for the carcinomas in this material was also analysed (Eneroth et al 1970) It was found that the muco-epidermoid carcinomas could be divided into two groups based on histopathology A total of 27 muco-epidermoid carcinomas was studied 21 of these were classified as highly differentiated In the 5–20 year follow up none of the 21 patients died from the tumour disease This finding demonstrates that highly differentiated muco-epidermoid carcinomas of low grade malignancy are not aggressive tumours and justifies local resectioning with suitable reconstruction procedures

Due to the low grade malignancy of the tumour in the present case a limited resection was planned After raising and stitching the flaps as has been described earlier in surgery of the cleft palate the risk of postoperative perforation was evident This complication is a major problem in reconstructive palate surgery and interferes with the rehabilitation of the patient Experience with fascia grafts in myringoplasty prompted us to use a free graft of fascia lata as a supporting measure The residual defect in the hard palate had to be filled with some substance stimulating a fibrosis for healing This is the same problem as in surgery of the skull base Elner & Koch (1974) have found that Surgicel® has a good capacity to stimulate fibrosis in skull base surgery For that reason we chose resorbable material for our case The long term follow up has demonstrated that a fibrosis could also be induced and the fibrous tissue acted as a skeleton for the mucosa growing from the border of the defect Half a year after operation there was no deformity in the hard palate thus indicating a rapid healing which is an advantage for the rehabilitation Surgicel® has also been used in the closure of non perforated defects in the hard palate At surgery of benign tumours large areas of bone and mucosa are often excised Application of Surgicel® in the de

created has always achieved complete healing without using local flaps.

In conclusion, a simple device for the strengthening of flaps in hard palate reconstructive surgery has been described. A modification of the method can be utilized in palatal non-perforated surgical defects

ZUSAMMENFASSUNG

Das Schließen von kleineren Defekten im harten Gaumen kann man durch lokale Schwenklambeaux beseitigen Es gibt immer ein Perforationsrisiko in dem Rekonstruktionsgebiet In diesem Falle war ein solches Risiko offenbar auf Grund eines großen und dunnen Lambeau im Operationsgebiet Um das Rekonstruktionsgebiet zu verstärken, wurden Surgicel® und Fascia lata angewandt Das postoperative Ergebnis war sehr gut, die Rehabilitierung des Patienten verlief ohne Komplikationen

REFERENCES

Elner, Å. & Koch, Hj 1974 Combined radiological and surgical therapy of cancer of the ethmoid *Acta Otolaryngol* (Stockh) *78* 270

Eneroth, C-M, Hjertman, L & Moberger, G 1970 Muco-epidermoid carcinoma of the palate *Acta Otolaryngol* (Stockh) *70* 408

Hjertman, L & Eneroth, C-M 1970 Tumours of the palate *Acta Otolaryngol* (Stockh), Suppl *263*, 179

A Biörklund, M D
University Hospital
S-221 85 Lund
Sweden

Acta Otolaryngol 82 151–156 1976

PROGRESS IN OESOPHAGOSCOPY

S Borgeskov, J Egedorf and H Kruse-Blinkenberg

From the Department of Thoracic Surgery Bispebjerg Hospital Copenhagen Denmark

(Received September 3 1975)

Abstract 667 endoscopic examinations of the oesophagus performed during a 5 year period by the rigid as well as by the flexible oesophagoscope are reviewed 80% of the procedures were diagnostic The examinations of 77 patients with oesophageal cancer stress the importance of performing simultaneous bronchoscopy on these patients In 10% the tumour was found to have invaded the bronchial tree On the basis of the biopsies taken there was a significantly better agreement with the final diagnosis when the biopsies were taken with a flexible than with a rigid oesophagoscope Owing to the simultaneous occurrence of gastric and duodenal diseases in 30% of the patients examination of the oesophagus should always be combined with gastro-duodenoscopy The importance of endoscopy in patients with dysphagia but negative X ray findings is stressed as a step towards earlier diagnosis of malignant oesophageal diseases Moreover a certain centralization of the examinations is recommended in consequence of the increasing expenses for modern endoscopic equipment and the special demands on its daily tending and maintenance

Endoscopic examination of the oesophagus may have either a diagnostic or a therapeutic aim It is performed on patients in whom X ray examination of the oesophagus shows changes needing confirmation by biopsy etc and on patients with normal X-ray findings, but with persistent symptoms indicating oesophageal disorder

In one third of patients with oesophageal diseases, Belsito & Dickinson (1972) found disagreement between the radiological and the endoscopic findings

Most often therapeutic oesophagoscopy is performed on patients with oesophageal strictures which have to be bouginaged and on patients presenting with foreign bodies in the oesophagus

The use of flexible endoscopes in diagnostic as well as therapeutic oesophagoscopy has by now largely replaced the conventional rigid instrument In consequence, increasing numbers of examinations are being performed under local anaesthesia Many different brands and types of flexible scopes are available A common feature is the construction of a highly flexible instrument in which the light (from an external source) as well as the picture are transmitted through coated glass fibres Experience in the present series is based upon the use of oesophagoscopes from the Olympus Optical Co, Japan The oesophagofiberscopes, 65 or 85 cm in length, are for straightforward viewing It is possible to insufflate air, flush water, and aspirate secretions through a channel 2 mm in diameter This channel also admits biopsy forceps brushes to collect cytological specimens, and forceps to remove foreign bodies (Fig 2)

Treatment of benign oesophageal strictures can be carried out with the Eder Puestow stringless dilators, with the wire guide passed through the biopsy channel (Price et al, 1974)

In the new models, controlled angular bending of the distal tip is possible, in four directions The four way wide angulation mechanism and the forward viewing optical system



Fig. 1 Flexible oesophagoscope of the brand Olympus EF and gastrointestinal fiberscope model GIF-K

make possible smooth insertion into the cavities for observation and photography. Using the two long models GIF D & K it is easy to loop the tip of the scope, and visualize the cardia from the stomach. This part of the examination is very important, mainly because of the frequent occurrence of malignant changes at the cardia. Now it is also possible to make a certain diagnosis of hiatal hernia (esp. the rolling para-oesophageal type) and incompetence of the hiatal sphincter (Anselm et al., 1971, Hradsky et al. 1973).

The ease of high-quality photography via the flexible scopes is of importance in reporting oesophageal findings, and in patients with oesophageal varices it can be of diagnostic value too. The reproduction of colours through the glass fibres is not perfect. The mucosa assumes an abnormal reddish yellow colour (Conn et al., 1967), and the slightly blue colour of varices cannot be perceived by the human eye. A film having a higher sensitivity to blue light, such as Kodak Ectachrome, can in such cases disclose the varices when the exposures from the examination are studied.

The most serious complication to endoscopic examination of the oesophagus is perforation of the organ. The frequency of instrumental perforations has been reduced by at least 50% since the introduction of the flexible instruments. With the rigid scope the frequency of perforations has varied in different series from 0.11% (Katz 1969) to 0.7% (Bell et al., 1956). In a series of some 40 000 endoscopic examinations Palmer & Wirts (1957) found 0.25% perforations and Elner & Dahlback (1962) 0.51% in 3964 cases. With a flexi-rigid scope Katz had 0.05% and with the flexible one 0.09%. Schiller et al. (1972) noted 0.05% perforations with the flexible scope.

The symptoms of oesophageal perforation are related primarily to the location of the injury. Almost all perforations are associated

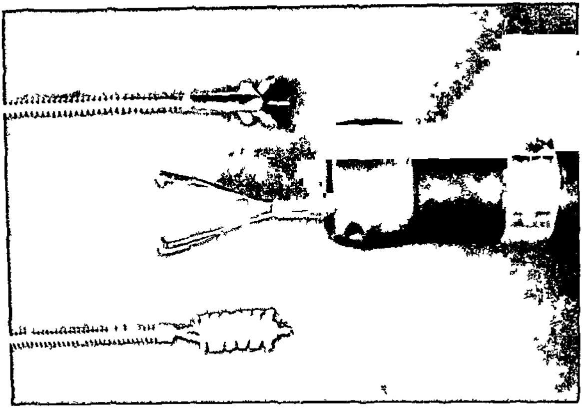


Fig 2 Biopsy forceps cytology brush and foreign body forceps for the flexible endoscope

with pain fever and dyspnoea The temperature and pulse rate rise and subcutaneous emphysema spreads from the suprasternal notch Treatment should be individualized but surgical management offers the most reliable results (Castberg 1965 Gerard et al 1968 Young & Nicoloff 1969)

MATERIAL

The present material comprises 667 endoscopic examinations of the oesophagus performed during the period 1969–1973 A total of 30? patients were examined thus on average each underwent 2 2 examinations 186 patients were males and 116 females Only 6 of the patients were under ?0 years of age 213 examinations were carried out by the conventional instrument 454 by a flexible oesophagoscope of the brand Olympus EF

Table I lists the most important diagnoses

Therapeutic oesophagoscopies (65) make up one fifth of the total consisting mainly in the bouginage of benign strictures

Fifty seven patients had hiatal hernia of the sliding type 25 complicated by peptic oesophagitis In 29 patients there was a question of peptic oesophagitis without a co-existing hernia i e reflecting pure cardial incompetence Nevertheless this was described as a finding in only 15 endoscopies

Bronchoscopy was carried out routinely in connection with the first oesophagoscopy in all patients with oesophageal stricture Among the 77 patients with oesophageal cancer invasion of the trachea or left main bronchus was found in 8 (10%) a sure sign of inoperability In another 22 patients bronchoscopy revealed abnormalities usually bronchitic changes due to overflow from the

Table I *Survey of diagnosis*

Diagnoses	No of patients
Benign stenosis	43
Malignant stenosis	77
Oesophageal diverticulum	20
Oesophageal hiatal hernia	57
Oesophageal perforation	8
Foreign body	3
Peptic oesophagitis	54
Corrosion	28
Cardiospasm	24
Oesophageal varices	2
Observation	62

phagus to the bronchial tree in patients with oesophageal stricture Among the 77 patients with oesophageal malignancy endoscopy revealed tumour tissue in 32, while in 45 cases it showed only stenosis, but the mucosa was intact, due to submucous growth of the tumour

In the course of the oesophagoscopies biopsies were obtained in 124 cases (Table II) Only 3 specimens were unsuited With the flexible instrument twice as many patients were biopsied as with the conventional endoscope Malignancy was diagnosed in 54 cases, benign conditions in 67

Table III presents the findings in the 77 patients with malignant lesions 42% had visible tumour tissue at endoscopy In 10 no biopsy was taken

Repeated biopsies or subsequent operation disclosed in 15 cases that a benign biopsy report had to be revised This is 22% ranging from 11 9% for biopsies removed with the flexible endoscope to 40% for those removed with the conventional instrument This difference is statistically significant ($\chi^2=5\ 57$ i e $0\ 05<p<0\ 01$) In two cases operation failed to disclose malignancy diagnosed on the basis of the biopsy (i e 1 6% false positives) The flexible and the conventional instrument contributed by one false positive each

Sixty-two of all the patients had oesophagoscopy without preceding X ray examination These were partly emergency cases with corrosion after the intake of acid or alkali partly patients with tumours of the lung who had the oesophagoscopy done in connection with bronchoscopy Among 240 patients who had been X-rayed, 84% (202) showed agreement between the X ray and endoscopic findings

A positive X ray finding was confirmed in 180 cases by endoscopy, while in 23 cases the endoscopy was negative Despite negative X-ray appearances, 15 patients (6%) showed signs of oesophageal disease at endoscopy In 3 of these cases the lesions were malignant the other were slight peptic stenosis and incompetence of the cardia

In 88 patients (30%) the radiological findings or the history indicated co-existent gastric or duodenal disease

No perforations occurred in the course of the 667 oesophagoscopies, but one in connection with bougienage following oesophagoscopy

DISCUSSION

An ever increasing number of oesophagoscopies are being performed with the flexible instrument, partly because the examination can then be done under local anaesthesia without major discomfort to the patient, partly because the risk of perforation is far less with this instrument than with the rigid oesophagoscope After the advent of applicable foreign body forceps and the possibility of performing bougie therapy by the flexible instrument the need for the conventional oesophagoscope is being restricted In cases of severe bleeding from the oesophagus and retention of food in patients with strictures

Table II *Distribution of biopsies taken with rigid and flexible oesophagoscope*

Histological diagnoses	Conventional equipment	Flexible equipment	Total
Malignant	16	38	54
Benign	21	46	67
Specimen unsuitable	1	2	3
No of biopsies	38	86	124

Table III *77 patients with malignant oesophageal disease*
No biopsy in 10 patients

	Tumour seen	No tumour seen	Total
	32	45	77
Flexible scope (42)			
Malignant diagn	18	19	37
Benign diagn	2	3	5 (11 9%)
Rigid scope (25)			
Malignant diagn	8	7	15
Benign diagn	2	8	10 (40%)

most doctors prefer the stiff oesophagoscope, and the passing of a pertubation tube by the method of Souttar also requires a special, rigid endoscope

Only a few of the present patients had hiatus hernia, and a more detailed analysis is not possible Only some of them were examined by a flexible endoscope, and only by the short type which does not permit retroflexion with examination of the cardia from the gastric aspect As a rule, biopsies of the mucosa were not taken, unless there was a suspicion of tumour Therefore, the recording of oesophagitis must be regarded with reserve Histological examination of oesophageal mucosa often shows inflammatory changes in the submucosa, even when the mucosa appears to be normal on endoscopy (Kobayashi & Kasugai 1974)

Chavy et al (1967) have called attention to the value of routine bronchoscopy on patients with oesophageal disease, in order to disclose invasion of oesophageal carcinoma into the trachea and bronchi 17% of the carcinomas infiltrated the trachea and bronchi ranging from 30% of the cervical to 6% of the low seated tumours This does not differ essentially from the present 10% The majority of these tumours were situated in the cardia and lower third of the oesophagus

The lacking agreement between the X ray and endoscopic findings in about one fifth of the patients is an experience also other materials For instance, Belsito & Dickinson (1972) found disagreement between the X-ray appearances and endoscopy in more than one third of their cases The discrepancy was due mainly to the numerous cases having oesophagitis but also to hiatus hernia and oesophageal varices

It applies to the conventional as well as to the flexible instrument that the majority of biopsies are applicable for histological evaluation, i e sufficiently large and not traumatized

In 22% of the malignant cases the first biopsy report was benign These false negatives could be traced in most cases to examinations performed with the conventional instrument With the latter 40% received a benign diagnosis as compared with 12% examined by the flexible instrument This significant difference is naturally explained by the great accuracy with which biopsies can be taken when using the flexible instrument However—considering the size of the biopsies—it is striking that this great difference between the two methods applies to the group of patients in whom the tumour tissue was not directly visible In these cases better results would have been expected with the larger biopsies obtainable by the conventional instrument but perhaps the result may be taken to indicate that the biopsies taken with the flexible scope are sufficiently deep despite the small size of the forceps

Two false positive diagnoses were recorded In one of these cases the pathologists maintain that the biopsy shows malignancy, but serial sectioning of the resected cardial area did not disclose further tumour tissue Accordingly, the false positive should perhaps sooner be recorded as "early cancer"

In retrospect, 88 patients (30%) ought to have had gastroscopy in connection with the oesophagoscopy These are patients with radiologically demonstrated duodenal or gastric ulcer, patients with corrosions (4 of 28 patients with oesophageal corrosion also [illegible] c corrosion), patients with hyper-

chlorhydria or achlorhydria and X ray nega tive patients with gastro-duodenal symptoms When also considering that hiatal hernia and cardial cancer require gastroscopy, to afford a possibility of a reasonable assessment, the indication for isolated examination of the oesophagus hardly exists any longer

Since, furthermore, the prospects of cure for patients with oesophageal cancer are so dismal, regardless of the treatment given, it would seem that the indication of oesophago gastroscopy would have to be extended to patients also with mild dysphagia and nega tive X-ray findings, in the attempt to institute treatment earlier in this category of patients in such cases the flexible scopes must be the method of choice, with the patient under local anaesthesia (Endo et al , 1971, Suzuki et al , 1972)

The relatively high cost of the instruments, and the necessity of possessing a suitable selection to ensure optimal examination, bring up the question of centralizing the diagnostic equipment within a few hospital units

ZUSAMMENFASSUNG

Die Ergebnisse von 667 endoskopischen Untersuchungen des Speiserohrs die während einer 5jährigen Periode mit flexiblem und/oder starrem Ösophagoskop durchgeführt wurden sind werden mitgeteilt 80% der Untersuchungen wurden zwecks Diagnose ausgeführt die übrigen in therapeutischer Hinsicht Die Untersuchungen von 77 Patienten mit Ösophaguskrebs deuten die Wichtigkeit einer gleichzeitigen bronchoskopischen Untersuchung an In 10% dieser Fälle hatten die Geschwüre die Trachea oder die Bronchien invadiert Mit der endgültigen Diagnose verglichen waren die Biopsien die durch flexible Skope entnommen wurden in größerer Übereinstimmung als diejenigen die durch starre Skope erreicht worden waren Als ungefähr ein Drittel der Fälle zu gleicher Zeit Zeichen einer Erkrankung des Ventrikels oder des Duodenums darstellten ist die Kombination von Ösophagoskopie mit Gastro-Duodenoskopie empfehlungswert Die endoskopischen Untersuchungen mit flexiblen Ösophagoskopen bei Patienten mit Schluckbeschwerden in denen aber die röntgenologische Untersuchung normal befunden ist wird [illegible]

[illegible]

Ösophagoskopie [illegible] nach der mit starrem Gerät weithin überlegen Die Kosten einer Anschaffung von flexiblen Skopen sowie die notwendige Sorgfalt der täglichen Verwendung und Erhaltung von diesen Instrumenten bietet eine gewisse Zentralisierung an

REFERENCES

Anselm K Schuman B & Priest R 1971 Fiberoptic esophagoscopy in geriatric patients *J Am Geriat Soc 19* 167

Bell J W Beskin C A & Starkey G W B 1956 Mediastinal abscess following instrumental perforation of the esophagus *Am J Surg 91* 999

Belsito A A & Dickinson P 1972 Fiberoptic esophagogastroscopy *Geriatrics 27* 90

Castberg T 1965 Traumatic perforation of the esophagus *Acta Chir Scand* Suppl *343* 56

Chavy A Vasselin M Fauvet J & Lemoine J M 1967 La trachéo-bronchoscopie de 700 malades porteurs de cancers de l'oesophage *Bronches 17* 370

Conn H O Binder H & Brodoff M 1967 Fiberoptic and conventional esophagoscopy in the diagnosis of esophageal varices *Gastroenterology 52* 810

Elner Å & Dahlback O 1962 Instrumental perforation of the esophagus *Acta Otolaryngol (Stockh) 54* 279

Endo M Kobayashi S Suzuki H Takemoto T & Nakayama K 1971 Diagnosis of early esophageal cancer *Endoscopy 2* 61

Gerard E P Sabety A M Trillo R A & Fernando M B 1968 Esophageal perforation *Arch Surg 96* 414

Hradsky M Stockbrügger R Wennerholm M & Östberg H 1973 Examination of the gastroesophageal area with the Olympus Fiberscope In *The function of the Esophagus* Odense University Press Odense Denmark

Katz D 1969 Morbidity and mortality in standard and flexibel gastrointestinal endoscopy *Gastrointest Endosc 15* 134

Kobayashi S & Kasugai T 1974 Endoscopic and biopsy criteria for the diagnosis of esophageal ... with a fiberoptic esophagoscope *Digest Dis 19* 345

Palmer E D & Wirts C W 1957 *Survey of gastroscopic and esophagoscopic accidents* *J Am Med Assoc 164* 2012

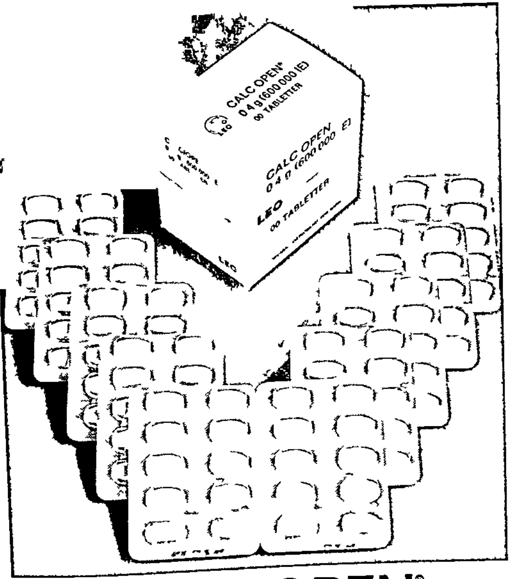
Price J D Stanciu C & Bennett J R 1974 A safer method of dilating esophageal strictures *Lancet I* 1141

Schuller K F R Cotton P B & Salmon P R 1975 The hazards of digestive fiber-endoscopy *Gut 16* 1027

Suzuki H Kobayashi S Endo M & Nakayama K 1972 Diagnosis of early esophageal cancer *Surgery 71* 99

Young J & Nicoloff D 1969 Management of esophageal perforation *Surgery 65* 764

S Borgeskov M D
Thorvaldsensvej 4 R
Rigshospitalet
Blegdamsvej 9
DK 2100 København
Denmark



CALCIOPEN®

V-penicillin baserat på klinisk forskning

Fó yt e ga e upp ysn nga Se FASS®

LEO • HELSINGBORG

Sinuiter

— *It should be noted that ampicillin, like phenoxymethylpenicillin, does not penetrate into sinus secretions from blood in reliable concentrations.* —

H. Gnarpe and C. Lundberg, Scand J. Inf. Dis 3 257, 1971

*

— Our investigation shows that doxycycline passes into the sinus secretions even under severe inflammatory conditions. —

C. Lundberg et al., Lancet II 107, 1968

*

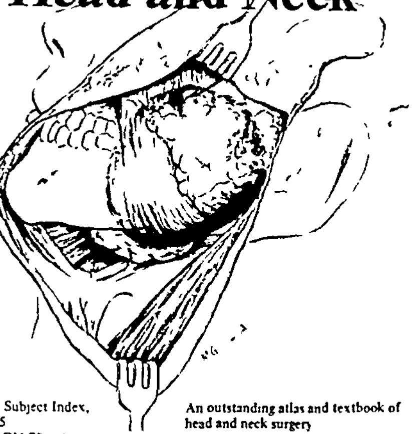
Vibramycin®

doxycyklin

en dos per dag

Pfizer

Pfizer AB, Täby



Acta Otolaryngol 8? 157–164 1976

THE UNMYELINATED NERVE FIBRES OF THE COCHLEA

A Paradiesgarten and H Spoendlin

From the Department of Otolaryngology Kantonsspital Zurich Switzerland

(Received November 30 1975)

Abstract A quantitative analysis was made of the unmyelinated nerve supply to the cochlea in normal cats and after various nerve lesions with different survival times The normal fibre distribution in the intraganglionic spiral bundle (IGSB) and the osseous spiral lamina (OSL) varies characteristically along the cochlear turns The unmyelinated nerve fibres are mainly associated with the IGSB and originate partly in the superior cervical ganglion and partly in the brain stem They seem to have an excellent capacity to regenerate

Although the unmyelinated nerve fibres greatly outnumber the myelinated fibres in the intraganglionic spiral bundle (IGSB) of the cat cochlea the precise origin distribution and function of these nerves is not as clearly understood as is that of the myelinated fibres This study was carried out (*a*) to quantify the unmyelinated fibres in the cochlea at different levels (*b*) to obtain information on their possible origin (*c*) to investigate the relationship of the unmyelinated fibres to the sympathetic nerve supply of the cochlea

METHOD

(*a*) Normal adult cat cochleas fixed in vivo and prepared according to the block surface technique described previously (Spoendlin & Brun 1974) were studied by light and electron microscopy The total number of myelinated and unmyelinated fibres was counted in the intraganglionic spiral bundles (IGSB) present in each representative radial section for every turn of the cat cochlea and in the osseous spiral lamina

(*b*) The degenerative progress of the nerve fibres in the intraganglionic spiral bundle (IGSB) was studied following selective nerve lesions Superior cervical ganglionectomy (sympathectomy) total vestibular nerve section (vestibular nerve) section of the cochlear nerve and inferior division of the vestibular nerve (VIII nerve) and section of the VIII nerve combined with superior cervical ganglionectomy (VIII+ symp) were performed The animals were sacrificed after survival times of 1–28 months and examined as stated in (*a*) Using the same point of reference in the upper basal turn in each animal comparison between the different lesions was possible

(*c*) To study the relationships of the unmyelinated nerve fibres to the sympathetic nerve supply of the cochlea the false transmitters 5 hydroxydopamine (5 OH DA) and 6-hydroxydopamine (6-OH DA) were employed Cats were pretreated with 5 OH DA intraperitoneally (4×20 kg over 48 hours) or 6-OH DA intravenously (50 mg/kg single dose 3 hours prior to fixation) 6-OH DA was also administered in a separate experiment by local perfusion through the opened round window (0 2 mg in 0 05 ml distilled water) for 5 minutes prior to fixation KMNO4 was used as a cochlear fixative in normal untreated cats to study its reported non specific enhancement of dense core vesicles in adrenergic nerve terminals (Densert 1974)

The cochleas were all prepared by the block surface technique and sectioned in both radia

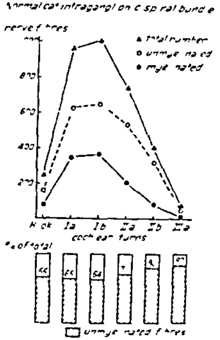


Fig 1 Normal fibre distribution in the intraganglionic spiral bundle of different cochlear turns and percentage of unmyelinated nerve fibres

and longitudinal planes The spiral ganglion and the IGSB, the osseous spiral lamina and habenula region and the organ of Corti were examined electron microscopically in all instances

RESULTS

(*a*) The IGSB is largest in the upper basal turn with a maximum total number of about 1 000 nerve fibres, which decreases gradually towards the base and the apex of the cochlea (Fig 1) In all turns the unmyelinated outnumber the myelinated fibres by a factor ranging from 2 1 in the basal turn to more than 6 1 in the apical turn, even though the total number of fibres involved at this level is less than in the basal turn (Fig 2)

The nerve fibre density in the osseous spiral lamina as determined by the number of fibres per 100 μ is largest in the lower basal turn The number of unmyelinated fibres, however, does not vary very much along the cochlea, except for a slight increase at the apex, but their percentage of the total number of all nerve fibres is smallest in the cochlear mid-portion and largest at the apex (Fig 3)

(*b*) In degeneration studies after various selective nerve lesions the following results were found in the IGSB (Fig 4)

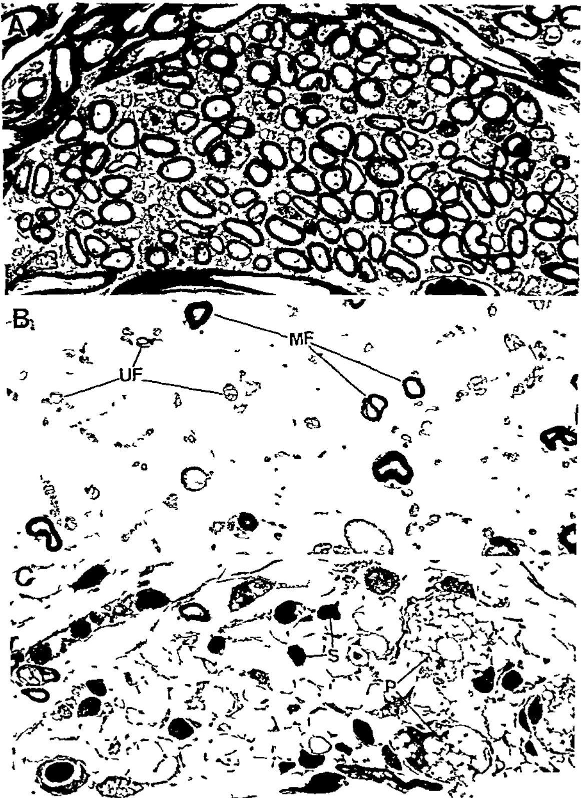
(i) A 50% reduction of the unmyelinated fibres occurs after extirpation of the superior cervical ganglion, whereas the myelinated fibres remain in normal numbers

(ii) A similar reduction of the unmyelinated and an almost complete disappearance of the myelinated fibres after sectioning of the vestibular nerve The same results are obtained if the lesion includes the cochlear nerve (Fig 2)

(iii) The greatest loss with almost complete disappearance of all myelinated and unmyelinated fibres occurs after a combined lesion of the superior cervical ganglion and the VIII nerve (Fig 2)

(*c*) Pretreatment with false transmitter agents showed a consistent rarity of reactive adrenergic nerve terminals in the cat Particular attention was paid to the organ of Corti, habenula region and tympanic lip vessels as well as IGSB Typical adrenergic terminals with dense core vesicles were found between the myelinated fibres just proximal to the habenular openings (Fig 5) Scattered dense core vesicles may also be present intra-axonally along the course of those nerves which are associated with adrenergic terminal swellings in the tympanic lip No accumulation of dense core vesicles was found distal to the habenula although isolated vesicles were found in the organ of Corti on occasion even in untreated cochleas No great accumulations were seen in the IGSB or

Fig 2 (A) Cross sectioned IGSB in a normal cat with myelinated (*MF*) and a great number of unmyelinated fibres (*UF*) of varying size (B) IGSB 8 months after section of the VIII nerve in the inner acoustic meatus Very few myelinated (*MF*) but a considerable number of unmyelinated fibres (*UF*) are found (C) IGSB 6 weeks after combined VIII nerve section and superior cervical ganglionectomy Mainly Schwann cells (*S*) and some phagocytes (*P*) are present but practically all myelinated and unmyelinated fibres have disappeared


A
B
UF
MF
C
S
P

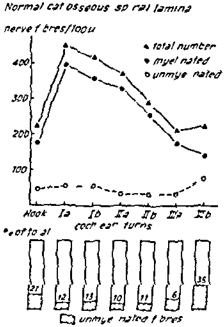


Fig 3 Nerve fibre densities in the distal part of the osseous spiral lamina and percentage of unmyelinated nerve fibres

around the tympanic lip vessel even though these structures were sectioned longitudinally to permit more adequate examination of longer *distances than is possible by radial sectioning alone*

KMNO4 performed poorly as a fixative and did not increase the number of adrenergic *terminals that could be shown by the more* specific agent

DISCUSSION

(*a*) Since the fibres of the olivo cochlear bundle enter the spiral ganglion at the level of the upper basal turn and expand from there as the IGSB it is not too surprising to find the maximum number of myelinated and unmyelinated fibres in the IGSB of the upper basal turn gradually decreasing in both basalward and apicalward direction At all points the unmyelinated component exceeds that of the myelinated efferent nerve fibres Although the total number decreases the percentage of unmyelinated fibres increases considerably towards the apex of the cochlea which could—but does not necessarily—mean that some of the myelinated fibres lose their myelin sheaths along their way through the IGSB (Maw, 1974) The great majority of the unmyelinated fibres however enter the cochlea as such coming as unmyelinated fibres from the brainstem with the vestibular nerve and through the anastomosis of Oort, where they can be demonstrated (Spoendlin 1972) Their possible origin might be the reticular formation as already indicated by Rossi & Cortesina (1963)

The two types of nerve fibres are not only distinguished by their sheaths but also by their relation to the Schwann cells and their average calibre All myelinated nerve fibres are individually surrounded by a single Schwann cell They show a variation in size (inside diameter) by a factor of 5 ranging from 0 6 μ to 2 6 μ The thickness of the myelin sheath varies from 0 15 μ to 0 6 μ The unmyelinated fibres, by contrast exhibit a greater degree of pleomorphism both in size and intra axional content The diameter ranges from 0 16 μ (small) to 1 6 μ (large)—a factor of 10 the majority of fibres being of the small or medium variety A single Schwann cell envelopes several different fibres simultaneously

It has been postulated (Maw 1974) that

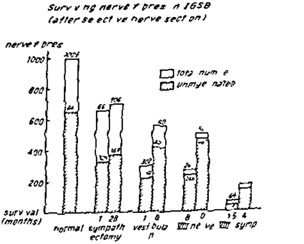


Fig 4 Relative reduction of myelinated and unmyelinated nerve fibres in the IGSB after various nerve lesions with short and long survival times

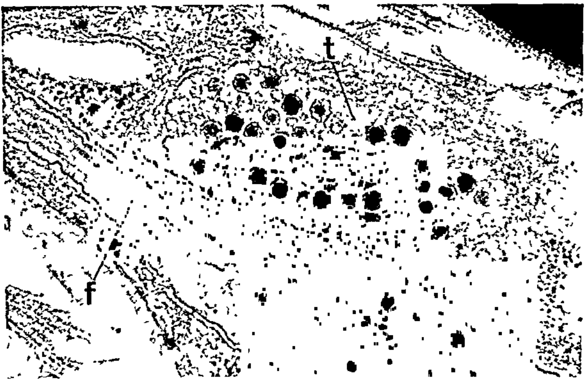


Fig 5 Adrenergic terminal swelling (*t*) and nerve fibre (*f*) between cochlear neurons prior to habenula with numerous dense core vesicles after pretreatment with 5 OH DA

some of the unmyelinated fibres terminate in the IGSB by synapsing with adjacent fibres However, in our material in longitudinal sections through the IGSB where the fibres could be followed over large distances, no evidence for such synapses was found It seems that the unmyelinated fibres, like their myelinated counterparts, merely travel through the IGSB toward their eventual destination, without any interaction between them along the way

In the normal osseous spiral lamina, a short distance prior to the habenula, the relationships of the number and ratio of nerve fibres are the reverse of that in the IGSB The myelinated fibres are in the great majority at this level, almost all of them being afferent in nature (Quantitative studies done after vestibular nerve sectioning show no statistically significant reduction in the resultant number of myelinated nerve fibres when compared with the normal osseous spiral lamina at the same level (Spoendlin, 1970)) The maximum total number of nerve fibres is once again in the basal turn The unmyelinated fibres, although in the minority throughout all turns, show an increase in their actual number in the apical turn, compared with the lower turns This is in contrast to the situation in the IGSB, where the total number of unmyelinated fibres is considerably reduced at the two extremes of the cochlea The increase in unmyelinated fibres in the osseous spiral lamina of the apex may be partially explained by the fact that the myelinated nerve fibres which normally lose their myelin sheath just prior to passing through the habenula may lose their sheaths a little sooner in the apical turn. Since the majority of the unmyelinated fibres are of much smaller calibre than the axons of the myelinated fibres, it is however more likely that they represent real unmyelinated fibres, which for some so far unknown reason are concentrated at the cochlear apex as ' h observed by Palumbi (1950)

(*b*) The loss of nerve fibres in the IGSB following selective nerve lesions shows very distinctive patterns Since the organ of Corti degenerates and disappears very rapidly following impairment of the cochlear blood supply the presence of the entire normal organ of Corti—excepting the neural component—is taken as a reliable sign that the blood supply was not impaired during surgical nerve lesions in the inner acoustic meatus Degeneration of the organ of Corti brings the suspicion that the blood supply was impaired by the surgical procedure which would lead to nerve degenerations unrelated to the lesion and to erroneous findings The occasional animal with a damaged organ of Corti was therefore excluded from the study

Spoendlin & Lichtensteiger (1966 1967) established that the sympathetic nerve supply to the ear is derived from two separate sources The blood vessel independent system (the larger denser and more pertinent to this study) arises from the ipsilateral superior cervical ganglion and enters the inner ear by a retrograde way through the middle ear terminating at the lip of the osseous spiral lamina The relatively less dense smaller component of blood vessel dependent fibres arises from the ipsi and contralateral stellate ganglion is directly associated with the blood supply and terminates at the level of the medium size vessels in the modiolus

The superior cervical ganglionectomy was found to cause a significant reduction in the number of unmyelinated fibres in the IGSB with up to 50% of these fibres being affected The myelinated fibres were unaffected by sympathectomy When the survival time following sympathectomy was prolonged the number of unmyelinated fibres surviving seemed to increase again slightly This is thought to be due to partial regeneration of some of the fibres which originate from remaining ganglion cells at lower levels in the cervical sympathetic chain not removed by the superior cervical ganglionectomy Wallerian degeneration of autonomic fibres is relatively slow as compared to the very rapid degeneration of somatic nerve fibres and appears to reach a maximum at 4–6 weeks

Sectioning the entire vestibular nerve or a combination of the inferior division of the vestibular nerve and the cochlear nerve gave similar results in the IGSB Naturally the addition of the cochlear nerve lesion caused degeneration of the spiral ganglion cell but had no effect on the IGSB itself which shows that the cochlear nerve does not contribute to the IGSB either with unmyelinated or with myelinated nerve fibres This is consistent with Spoendlin s (1972) earlier findings that there is a virtual absence of unmyelinated nerve fibres in the inner acoustic meatal portion of the cochlear nerve and that unmyelinated fibres occur in large numbers in the internal auditory meatal portion of the vestibular nerve the anastomosis of Oort and in the facial nerve With such nerve lesions a maximum of 2/3 of unmyelinated and 4/5 of myelinated fibres disappeared after a brief survival of one month With longer survivals there was a considerable increase again in both types of fibre in keeping with the postulate of fibre regeneration The myelinated and unmyelinated fibres almost double in number within a few months after they have reached a minimum at about one month following nerve section (Fig 4)

Combining the lesions of the VIII nerve and the superior cervical ganglion led to an *additive effect for the unmyelinated* fibres Maximum loss of these fibres occurred after such combined lesions and exceeded by far that which occurred after any single lesion alone whereas the reduction in myelinated fibres was comparable to the results after VIII nerve sections alone It is therefore probable that two different populations of unmyelinated fibres are involved with each single lesion and that both of these nerve populations are involved in the combination It is important to note however that contrary to theoretical expectation it was never possible to entirely eliminate either the myelinated or unmyelinated fibres Those fibres that did survive were

mostly of medium size Their origin remains to be clarified

The total number of unmyelinated fibres entering the cochlea seems to be very small compared to the myelinated fibres and most of them enter the spiral ganglion with the fascicles of the olivocochlear bundle in the upper basal turn Tangential sections through the spiral ganglion proximal to the level of the IGSB showed virtual absence of the unmyelinated fibres between the uniformly sized myelinated axons of the type I cochlear neurons The few scattered unmyelinated axons of the type II ganglion cells which Spoendlin (1971, 1972) has shown to comprise about 5% of the total number of spiral ganglion cells

From all these findings it is concluded that the unmyelinated fibres to the cochlea travel by way of the IGSB after they have entered the cochlea They partially originate in two distinguished separate groups of populations although these cannot be differentiated in the periphery One group takes its origin from the ipsilateral superior cervical ganglion, is independent of the blood vessels and enters the cochlea direct, as described previously (Spoendlin & Lichtensteiger, 1967) The other group arises in the brainstem and is associated with the vestibular nerve Whether or not the latter is part of the olivocochlear bundle is not immediately apparent The result of a selective lesion of the contralateral olivocochlear fibres in the floor of the 4th ventricle of the *brainstem (midline lesion) seems to indicate* that the resultant loss of fibres in the vestibular nerve is mostly myelinated (somewhat different findings were reported for the guinea pig by Terayama & Yamamoto, 1971), which would indicate a predominantly ipsilateral origin for the unmyelinated component But it is not known at the present time whether this component is related to the ipsilateral olivocochlear tract or originates in another nucleus

(c) The distribution and termination of the unmyelinated fibres beyond the IGSB is not very clear or obvious Iurato et al (1971) have shown histochemically that acetylcholinesterase (ACHE) is widely distributed in the cochlea both in the spiral ganglion and in the organ of Corti ACHE, however, is non-specific to any one neural system and can be found at all levels of the nervous system, in the adrenergic as well as in the cholinergic system

Noradrenalin on the other hand, a specific neuro-transmitter, is found exclusively in post ganglionic adrenergic nerve endings and as such is a much more valuable target for study It has been shown that the false transmitter substances 5 OH-DA and 6-OH-DA can be used to characterize the adrenergic post ganglionic nerve terminals at an ultrastructural level (Tranzer & Thoenen, 1967, Tranzer & Richards, 1971, Densert, 1974) 5 OH DA (ip or iv) leads to a selective accumulation of the metabolite in the adrenergic endings apparent in dense core vesicles 6-OH DA (iv or by local perfusion) leads to a selective destruction of the adrenergic endings, although this occurs only if allowed to act for a critical period of time prior to which it accumulates at first in the terminals in a way similar to 5-OH DA

Where, after pretreatment with one of these agents, the nerve terminal is still capable of permitting clear classification it is generally accepted that a homogeneous population of many small empty vesicles is indicative of a cholinergic terminal Dense core vesicles, varying in size but mainly small and only occa*sionally large, combined with interposed small* empty vesicles is indicative and characteristic of adrenergic terminals Dense core vesicles may also be found within adrenergic axons

The fact that only very few adrenergic terminals were identified in the habenular region of the cat—in contrast to other animals—may indicate that either the cat has fewer adrenergic fibres as compared with the rabbit (Densert, 1974) or that fewer of these adrenergic endings are in the active state at any one time Only isolated dense core vesicles were found in the organ of Corti and no accumulations of adrenergic vesicles were seen i

IGSB, which is in keeping with the findings of Spoendlin & Lichtensteiger (1966)

All this still leaves a great number of un myelinated fibres unaccounted for as regards distribution, termination and probable function It may be that more of these fibres (which may correspond to those that are associated with the vestibular nerve) may belong to a cholinergic efferent cochlear innervation

ACKNOWLEDGEMENT

A Research Fellowship from the Ontario Ministry of Health enabled A Paradiesgarten to participate in this study

ZUSAMMENFASSUNG

Die vorliegende Arbeit ergibt eine quantitative Analyse der unbemarkten Nervenfasern in der Cochlea normaler Katzen nach verschiedenen Nervenlasionen und verschiedenen Ueberlebenszeiten Die normale Faserverteilung im intraganglionaren Spiralfaserbundel und in der Lamina spiralis ossea andert in charakteristischer Weise von der Basis bis zur Schneckenspitze Die unbemarkten Fasern sind im wesentlichen mit den intraganglionaren Spiralfaserbundel assoziert und haben ihren Ursprung teils im Ganglion cervicale superius des cervikalen Sympathicusstranges und teils im Hirnstamm Sie scheinen eine starke Regenerationsfahigkeit zu besitzen

REFERENCES

Densert O 1974 The effect of 6-hydroxydopamine on the rabbit cochlea *Acta Otolaryngol* (Stockh) *78* 1

— 1974 Adrenergic innervation in the rabbit cochlea *Acta Otolaryngol* (Stockh) *78* 1

Iurato S 1962 *Efferent fibres to the sensory cells of* Corti s organ *Exp Cell Res 103* 162

Iurato S Luciano L Pannese E & Reale E 1971 *Histochemical localization of acetylcholinesterase* (ACHE) activity in the inner ear *Acta Otolaryngol* (Stockh) Suppl *279*

Maw, A R 1974 Synaptic contacts within the intra ganglionic spiral bundle and other recent ultra structural findings in relation to concepts of efferent cochlear innervation *Ann Otol Rhinol Laryngol 83* 180

Palumbi G 1950 Particolare apparato nervoso recettore nella regione apicale della chiocciola dell orecchio humano *Boll Soc Ital Biol Sper 26* 136

Ross M D 1971 Fluorescence and electron microscopic observations of the general visceral efferent innervation of the inner ear *Acta Otolaryngol* (Stockh) Suppl *286*

Spoendlin H 1970 Structural basis of peripheral frequency analysis In *Frequency analysis and periodicity detection in hearing* (ed R Plomb & S Smoorenburg) pp *1–40* Sijthoff Leyden

— 1972 Innervation densities of the cochlea *Acta Otolaryngol* (Stockh) *73* 235

— 1973 Autonomic nerve supply to the inner ear In *Vascular disorders and hearing defects* (ed Darin de Lorenzo) pp 93–111 University Park Press Baltimore/Maryland USA

Spoendlin H & Lichtensteiger W 1966 The adrenergic innervation of the labyrinth *Acta Otolaryngol* (Stockh) *61* 423

— *1967 The sympathetic nerve supply to the inner ear Arch Klin Exp Ohren Nasen Kehlkopfheilkd 189* 346

Terayama Y & Yamamoto K 1971 Olivo-cochlear bundle in the guinea pig cochlea after central trans section of the crossed bundle *Acta Otolaryngol* (Stockh) *72* 385

Tranzer J P & Richards J G 1971 Fine structural aspects of the effect of 6-hydroxydopamine on peripheral adrenergic neurons In *6 Hydroxydopamine and Catecholamine Neurons* (ed T Malmfors & H Thoenen) pp 15–31 North Holland Publishing Company Amsterdam

Tranzer J P & Thoenen H 1967 Electronmicroscopic localization of 5 hydroxydopamine (3 4 5 trihydroxy phenyl ethylamine) a New false sympathetic transmitter Separatum *Experientia 23* 743 Birkhauser Verlag Basel (Schweiz)

A Paradiesgarten M B F R C S
Mount Sinai Hospital
Toronto
Canada

Acta Otolaryngol 82 165–171, 1976

SELECTIVE HABITUATION OF VESTIBULAR NYSTAGMUS BY VISUAL STIMULATION IN THE MONKEY

L R Young[1] and V S Henn[2]

From the [1]Man-Vehicle Laboratory the Department of Aeronautics and Astronautics Massachusetts Institute of Technology Cambridge Mass USA and the [2]Department of Neurology Kantonsspital Zurich Switzerland

(Received April 21 1975)

Abstract Four monkeys were exposed to a series of uni directional rotating visual fields of the type known to produce circularvection in man As in the human case the vestibulo-ocular reflex for rotation in the direction of the optokinetic nystagmus and circularvection during the preceding visual stimulation—was markedly decreased following that visual stimulation whereas that in the opposite direction was increased This finding cor responds to the results of nystagmus and psychophysi cal circularvection experiments on man It suggests a link between single unit studies performed under identical conditions in monkey and psychophysical investigations

Exposure of humans to a large field with many high contrast borders moving at a uniform angular velocity induces a sensation of self motion (circularvection or linearvection) re sembling that associated with true body mo- tion Human vestibular nystagmus stimulated by passive body rotation in the dark is selec tively diminished in the direction of circular- vection following repeated exposure to uni directional visual field rotation inducing uni directional circularvection velocity sensations (Young & Henn 1974) To investigate the underlying neurophysiological basis of the interaction of visual and vestibular stimuli in the generation of nystagmus recordings were made from single units in the brain stem of awake unanesthetized monkeys using similar combinations of visual and vestibular stimula tion (Henn et al , 1974) The similarity be- tween the consistent occurrence of changes of vestibular nucleus unit firing rate in the monkey and circular vection in the human when identical stimuli were applied suggested the involvement of the vestibular nucleus in the integration of visual and vestibular motion cues both for sensation and nystagmus It remained however, to demonstrate that the response to moving visual fields in the monkey showed a behavioral similarity to that in humans We tested the hypothesis that mon keys, just as humans, would experience a uni directional decline in the vestibulo-ocular reflex following repeated exposure to motions of moving stripes which would induce circular- vection in humans

METHODS

Four awake unanesthetized monkeys were tested in the current study The first 3 were juvenile Rhesus monkeys (*Macaca mulatta*) The fourth (monkey number 5) was an African green monkey (*Cercopithecus aethiops*) Im- planted bolts in a skull holder held the head rigidly in a position pitched 25° forward, plac- ing the horizontal semicircular canals roughly in the horizontal plane Details of the method are given in Henn et al (1974) Animals were

This research was supported by NASA Grant NGR 22 009 701 NSG 2032 and by the Roche Research Founda tion

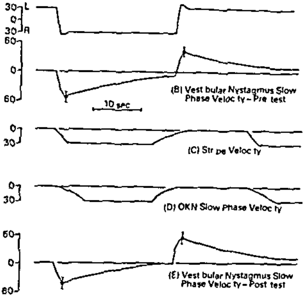


Fig 1 Test protocol and average pre and post OKN vestibular nystagmus responses All velocities in deg/sec (A) Table angular velocity showing first complete cycle of 60 deg/sec velocity changes Initial direction of tests randomized (B) Sketch of vestibular nystagmus slow phase velocity for pre test in dark showing average peak velocities and standard error of means for 4 monkeys Note initial directional preponderance for right accelera tion (C) Sketch of two of thirty repetitions of left moving *rotating stripe waveforms (D) Sketch of typical (right* beating) optokinetic nystagmus slow phase velocity as sociated with each moving stripe stimulus (E) Post visual stimulus vestibular nystagmus as in B showing decrease in vestibular nystagmus slow phase velocity for accelera tion *right* (nystagmus in the direction of the OKN) and in crease for acceleration *left*

not selected for vestibular response symmetry Alertness was maintained by injection of a light dose (roughly 0 1 mg/kg hourly) of amphetamine Horizontal eye movements were recorded using chronically implanted d c electrodes An analog differentiator with *20 ms time constant was used for calculation* of slow phase velocity of nystagmus

The vestibulo ocular reflex was tested in the dark before and after *exposure to 30 unidirec*tional optokinetic stimuli The test protocol and major results are shown in Fig 1 The monkeys were mounted on a servo controlled turntable kept covered with a light proof en closure for vestibular testing The table was driven about the vertical axis, usually for three complete cycles of sudden 60°/sec changes of *angular velocity for pre- and post test OKN* vestibulo-ocular testing Constant velocity (30°/sec) periods were nominally 25 sec The first complete cycle of 60° per second velocity steps to the right and left was used in data analysis In addition to minimize order effects, the averages of all right and of all left steps were used The peak angular velocity of slow phase *nystagmus, usually occurring* within the first 1 second following the change in velocity of the table, was measured from *the differentiated eye movement recording* The eye movement record and differentiator were calibrated from optokinetic nystagmus angular velocity records taken during long duration low velocity monkey rotation in the light before and after the main experiment The vestibulo-ocular gains (defined as the ratio of the peak slow phase velocity to the change *in* table angular velocity) were calculated from the pre- and post test stimuli in the direction of the intervening optokinetic *nystagmus and opposite to it, and the relative* change in gains taken as the data for further analysis The effect of any spontaneous nystagmus was not taken into account *in cal*culation of the vestibulo ocular gain

The visual stimulation was similar to that used for human experiments Vertical stripes were projected onto a cylindrical screen by a Tonnies moving shadow optokinetic pattern generator The screen was 2 m high and located 72 cm from the monkey, and covered 180° of the visual field Twelve light stripes, each 7 5°, were projected on the screen Angular velocity was controlled by a variable *speed motor* The stimulus was 30 repetitions of a unidirectional stripe pattern which, in hu mans at least, induced a feeling of circular *vection which built up to a constant angular* velocity and then decayed The stripe velocity was increased for a period of 5 to 10 sec from *0 to 30°/sec and then, after approximately* 30 sec of constant angular velocity, reduced back to 0°/sec over a period of approximately 5 sec Contrast of the *display was adjusted un*

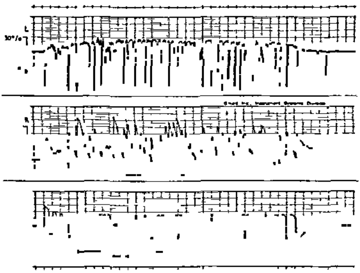


Fig 2 Development of OKN during one of thirty consecutive stimuli Top trace eye velocity middle trace horizontal eye position bottom trace OKN stimulus counter—each pulse indicates passage of field 15 deg Monkey 5 stripes moving left

til the animals showed vigorous optokinetic nystagmus (In the human experiments reported optokinetic nystagmus was inhibited by the use of a fixation point) Ten to fifteen seconds separated successive optokinetic stimuli

RESULTS

Each of the 4 monkeys produced optokinetic nystagmus under the stimulus of the moving stripes The strength of the nystagmus varied with the contrast Maximal optokinetic nystagmus was usually stimulated with a dimly lighted pattern rotating in a dark room High contrast bright stripes often failed to induce optokinetic nystagmus perhaps because the irregularities in the screen, visible to the experimenters served as fixation points for the monkeys Optokinetic nystagmus developed slowly during each visual stimulus trial and decayed to zero as the stripes were slowed to a stop as shown in Fig 2 (When the lights were suddenly extinguished on occasion optokinetic after nystagmus (OKAN) decayed over a period of approximately 20 sec)

No evident pattern of enhancement or fatigue of the optokinetic response during the thirty trials was observed Following the thirty OKN trials when the animal was placed *in the dark but not rotated* a spontaneous nystagmus in the direction opposite to the previous OKN generally developed and lasted for periods from several minutes to greater than half an hour

The vestibulo-ocular reflex gain compared before and after the OKN stimulus, shows a directionally selective transfer of habituation from visual stimulus, just as is observed in man Typical vestibular nystagmus on acceleration to the right and then to the left in the pre test prior to the "stripes left (right beating) OKN stimuli is indicated in Fig 3*a* Following the exposure to left moving stripes, however, the right beating vestibulo-ocular reflex is markedly diminished in magnitude and duration, whereas the left beating nystagmus is increased over the pre test values The order of testing of the vestibulo-ocular reflex was not a significant factor in the results

The vestibulo-ocular reflex gains for each monkey, for acceleration in and opposite to the direction of the intervening OKN and for both directions of OKN stimulus are tabulated in Table I In six of the eight cases for acceleration in the direction of the conditioning OKN gain decreased following visual stimulation considering first rotations al[illegible] and [illegible] for all tests averaged The mean de[illegible] was 18 7% for the first rotation [illegible] for the averages the only [illegible]

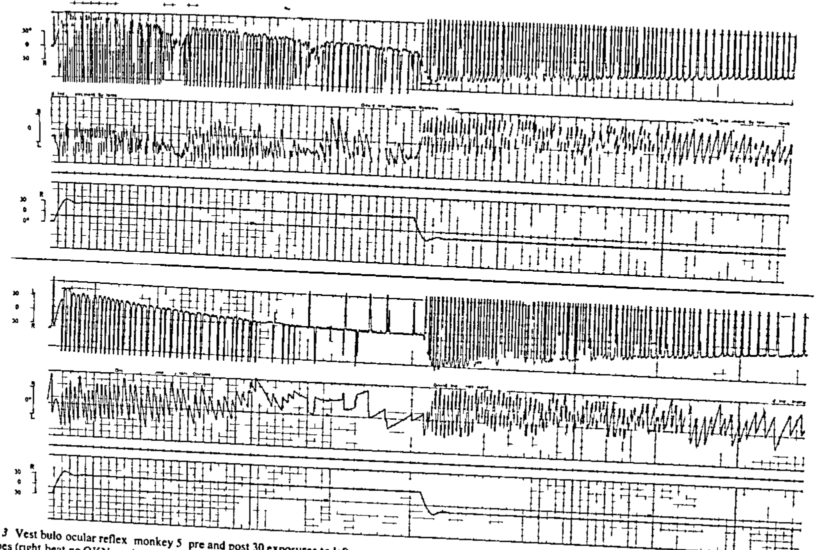


Fig. 3. Vestibulo-ocular reflex, monkey 5, pre and post 30 exposures to left moving stripes (right beating OKN as shown in Fig. 2). (A) First trials of 60 deg/sec step to right followed by 60 deg/sec step to left prior to OKN stimuli. Upper and middle traces as in Figure 2. Lower trace: table angular velocity stepping between 30 deg/sec left and right. (B) Same as A following 30 right beating OKN exposures. Notice the reduction in right beating vestibulo-ocular reflex peak velocity, frequency and direction, the occurrence of left beating nystagmus toward the end of the right turning, and the increased strength and duration of the response to the step to the left.

Table I a *Vestibulo-ocular reflex gain (peak slow phase nystagmus velocity/rotation velocity step) pre and post OKN stimulation based on first trial in each direction*

Monkey number	OKN direction	Vestibulo-ocular reflex gain in direction of OKN Pre OKN	Post OKN	(% decline)	Vestibulo-ocular reflex gain in direction opposite OKN Pre OKN	Post OKN	(% decline)	Difference in % decline of gain directions with OKN-opposite OKN
2	L	0.54	0.37	31.5	0.40	0.34	15.0	16.5
	R	0.40	0.29	27.5	0.40	0.51	−27.5	55.0
3	L	1.04	1.10	−5.7	0.99	1.04	−5.1	−0.6
	R	0.79	0.66	16.5	0.79	0.69	12.7	3.8
4	L	0.53	0.30	43.4	0.40	0.55	−37.5	80.9
	R	0.86	0.67	22.1	0.38	0.82	−115.8	137.9
5[a]	L	1.10	1.24	−12.7	1.14	1.33	−16.7	4.0
	R	1.48	1.08	27.0	1.05	1.59	−51.4	78.4
Average L and R				18.7			−28.3	47.0[b]

[a] African green monkey
[b] Difference significant $p<0.05$

minor.[1] On the other hand, for accelerations opposite to the OKN, the vestibulo-ocular reflex gain increased in 6 of the 8 cases, by an average of 28.3% for first rotations and 18.9% for the averages. The average difference between vestibulo-ocular reflex change with and opposite to the direction of the intervening OKN was 47% greater in the direction of the OKN for first rotations and 37.9% greater for the averages. The differences were positive for all but one first rotation comparison in which it was only 0.6% negative. These differences in the correlated means are significant ($p<0.05$, $t=2.67$, $n=8$, for the first rotations. $p<0.05$, $t=2.38$, $n=8$ for the averages). The duration of this transfer of habituation is short and easily abolished. It was not noticeable on testing the following day.

DISCUSSION

It is hardly surprising to find that a type of visual stimulus which produces circularvection and OKN in man, also produces OKN in monkeys. It is also to be expected that the effect of this visual stimulus in transferring to a unidirectional decrease in vestibulo-ocular reflex gain, observed in man, is also observed in the monkey. Many studies have been conducted on the transfer of habituation (Collins, 1975, Hood & Pfaltz, 1954, Miyoshi et al. 1973, Tibbling, 1970). We have previously argued that the necessary condition for the transfer of habituation to vestibular nystagmus is the generation of a repeated and consistent motion sensation, rather than nystagmus generation *per se* (Young & Henn, 1974, Pfaltz & Kato, 1974). The current results suggest that the monkey is experiencing something akin to circularvection during this visual stimulation, and that neural pathways from the visual system to areas responsible for motion sensation and for the generation of vestibular nystagmus would be affected. Our observations on the effect of moving visual fields on vestibular nucleus units in the monkey (Henn et al. 1974) together with those of Dichgans et al. (1973) in the goldfish are entirely consistent with this view.

Recent neurophysiological work on visual and vestibular influences in the generation of

[1] Monkey 5 showed a 13% increase in first rotation vestibulo-ocular gain for acceleration following left OKN trials. For both OKN directions, however, the duration of post rotation nystagmus was shortened in the direction of OKN and a secondary phase appeared.

Acta Otolaryngol 82 172–181, 1976

TREATMENT POLICY FOR MAXILLARY SINUS CARCINOMA

S Sakai,[1] H Fuchihata[2] and Y Hamasaki[2]

From the [1]Departments of Otolaryngology and [2]Radiology Osaka University Medical School Osaka Japan

(Received November 27 1975)

Abstract Some 908 cases of malignant tumors of the nose and paranasal sinuses treated from 1957 through 1974 were statistically analysed The most common tumor site was the maxillary sinus (91 4%) and the most common histological figure was carcinoma (92 4%)

The crude and relative survival rates for each treat ment mode were calculated in January 1975 The number of cases and the 5 year relative survival rates of the main groups were as follows

I Primary cases of malignant tumors (761 cases)	29 2%
A Carcinoma (709 cases)	29 3%
B Sarcoma (45 cases)	27 4%
II Primary cases of maxillary sinus carcinoma (561 cases)	26 1%
A Period 1957–66 (282 cases)	22 8%
1 Combination of irradiation and surgery (114 cases)	36 9%
2 Irradiation alone (168 cases)	12 7%
B Period 1967–69 (130 cases)	34 5%
1 Irradiation with 5 FU intra arterial infusion (25 cases)	36 1%
2 Irradiation with intra arterial infusion of other radiosensitizers (35 cases)	35 7%
3 Irradiation only without infusion (45 cases)	35 2%
C Period 1970–71 Linac X ray irradiation (61 cases)	15 9%
D Period 1972–73 Irradiation with 5 FU in fusion (80 cases) 3 year relative sur vival rate	39 3%

The stage grouping of maxillary sinus carcinoma based on the classification of tumor spread in the TNM system was recommended for the comparison of survival rates The best mode of treatment in our experience is the combina tion of Co-60 γ-ray irradiation and continuous intra

Maxillary cancer is found more frequently in Japan than anywhere else in the world The incidence of maligant tumors of the nose and paranasal sinuses is almost the same as that of laryngeal cancer in this country Maxillary cancer is one of the most difficult tumors to treat in the field of otolaryngology Although we have observed a gradual improvement of therapeutic results for this cancer in recent years thanks to new developments in radiation therapy and local chemotherapy, the long term result in our large series falls far short of our expectations Careful planning of ran domized studies and statistical comparison of long term results are indispensable in the search for the best treatment method for this cancer

908 cases of malignant tumor of the nose and paranasal sinuses treated in the period 1957–74 were statistically analysed The primary sites and the histological diagnoses of the tumors are shown in Table I The most common site was the maxillary sinus (91 4%) and the most

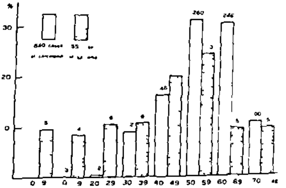


Fig 1 Age distribution of patients with malignant tumors of the nose and paranasal sinuses

Table I *Primary sites and histological diagnoses of malignant tumor of the nose and paranasal sinuses (1957–74)*

	Maxil sinus	Ethmo cells	Front sinus	Sphen sinus	Nasal cavity	Other	Total	
Carcinoma (total)	792	29	8	0	11	0	840	925
Squamous cell ca	549	13	2	0	4	0	568	626
Adenocarcinoma	24	3	0	0	1	0	28	031
Adenoid cystic ca	10	2	0	0	1	0	13	014
Mucoepidermoid ca	5	1	1	0	1	0	8	009
Anaplastic ca	30	6	1	0	2	0	39	043
Transit cell ca	6	1	0	0	1	0	8	009
Carcinoma NOS	168	3	4	0	1	0	176	194
Malignant melanoma	1	1	0	0	9	0	11	012
Sarcoma (total)	37	3	0	1	9	5	55	061
Fibrosarcoma	7	0	0	0	1	0	8	009
Liposarcoma	2	0	0	0	0	1	3	003
Rhabdomyosarcoma	8	0	0	0	0	1	9	010
Osteosarcoma	2	0	0	0	0	1	3	003
Chondrosarcoma	3	1	0	0	0	0	4	004
Ewing s tumor	0	0	0	0	0	1	1	001
Reticulum cell sa	11	2	0	1	8	0	22	024
Plasmacytoma	1	0	0	0	0	0	1	001
Sarcoma NOS	3	0	0	0	0	1	4	004
Olfact neuroblast	0	0	0	0	2	0	2	002
Total	830	33	8	1	31	5	908	
	914	036	009	001	034	006		1 000

common diagnosis was carcinoma (92 5%) The male/female ratio was 3 2 The age distribution of the patients with carcinoma and sarcoma is shown in Fig 1 Carcinoma was most prevalent in the fifties and sixties, while the sarcoma affected younger age groups

The crude survival rates of all primary cases (the cases with no previous treatment) and the secondary cases (previously treated cases minus those apparently healed at the time of the first examination) are shown in Fig 2 The 5 year relative survival rate for the primary cases of malignant tumor of the nose and paranasal sinuses was 21 3%

The TNM classification of maxillary cancer has not been agreed upon by UICC The author proposed a TNM classification in 1967 and 1971 (Sakai 1967, 1971) The following stagegrouping appeared to be a reasonable one from the prognostic viewpoint

Stage I	T1–2	N0	M0
Stage II	T3	N0	M0
Stage III	T4	N0	M0
	T1–4	N1	M0
Stage IV	T1–4	N2–3	M0
	T1–4	N0–3	M1

Table III shows the 3-year crude survival rates according the age, sex and author's NTM

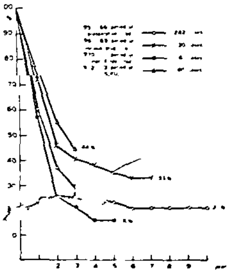


Fig 2 Relative survival rates for different treatment policies (1957–73)

Table II *Crude survival rates of malignant tumor of the nose and paranasal sinuses (statistics as of January 1975)*

	1 year	2 years	3 years	4 years	5 years
Primary cases[a]	443/761 583	251/723 347	179/667 268	145/623 233	121/569 213
Carcinoma	415/709 585	234/675 347	167/623 268	136/584 233	114/533 214
Sarcoma	25/45 556	14/41 341	10/38 263	8/34 235	6/31 194
Malignant melanoma	2/6	2/6	1/5	0/4	0/4
Olfactory neuroblast	1/1	1/1	1/1	1/1	1/1
Secondary cases[b] minus apparently healed cases at the time of the first examination	20/69 290	11/64 172	7/59 119	5/54 093	4/51 078

[a] Previously untreated cases
[b] Previously treated cases

classification and staging The 3-year crude survival rates for Stages I, II, III, IV were estimated at approximately 45, 25, 15 and 0%, respectively The survival rate became poor with age but did not differ significantly between the sexes It is apparent from Table III that two important prognostic factors—the age of the patient and the stage of disease—should be taken into careful consideration when planning the clinical study and also in the statistical analysis of data obtained

Our criteria of treatment effect are as follows

A The criteria of clinical effect
- Degree 1 Tumor unchanged or enlarged
- Degree 2 Tumor regressed
- Degree 3 Tumor disappeared but covered with necrotic tissue Residual tumor suspected
- Degree 4 Tumor disappeared and covered with thick white coat
- Degree 5 Tumor disappeared and epithelialization seen

A tumor was considered to have disappeared clinically (clinical tumor clearance rate) in Degrees 4 and 5

B The criteria of microscopic effect
- Degree 1 No effect of treatment
- Degree 2 Many tumor cells seen
- Degree 3 A few tumor cells seen
- Degree 4 Only degenerated tumor cells seen
- Degree 5 No tumor cells seen

A tumor was considered to have disap

Table III *Three year crude survival rates according to sex, age and clinical stage at the time of the first examination*

Sex		
Male	87/301	289
Female	53/190	279
Age		
0–39	18/53	340
40–49	34/89	382
50–59	50/159	314
60–69	29/138	210
70–	9/52	173
TNM Classification		
T1 N0 M0	3/5	600
T2 N0 M0	54/124	435
T3 N0 M0	70/257	272
T4 N0 M0	8/55	145
T2 N1 M0	1/5	200
T3 N1 M0	3/21	143
T4 N1 M0	1/14	071
T1–4 N2 M0	0/7	000
T1–4 N3 M0	0/2	000
T1–4 N0–3 M1	0/1	000
Stage grouping		
Stage I	57/129	442
Stage II	70/257	272
Stage III	13/95	137
Stage IV	0/10	000
Total	140/491	285

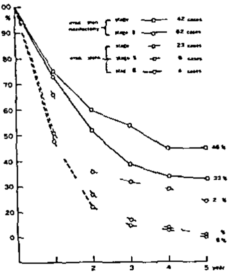


Fig 3 Relative survival rates according to treatments and clinical stages (1957–66) Patients of more than 70 years of age are excluded

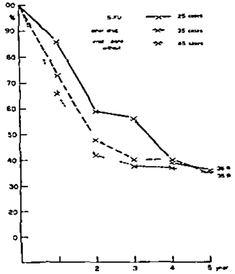


Fig 4 Relative survival rates according to different treatment modes (1967–69)

peared microscopically (microscopic tumor clearance rate) in Degrees 4 and 5

C Local non recurrence rate

Local non recurrence rate was calculated at such set intervals as every half year Those patients who expired during a certain period due to metastasis or other causes with no evidence of local recurrence were excluded from the calculation of the local non recurrence rate during that period

D Survival rate

The crude survival rate was computed according to the TNM General Rules of IUCC (1974) The cumulative survival rate was computed by actuarial method (Cutler 1958 Schultz 1969 UICC 1974) The relative survival rate is formulated as (Ederer 1961)

$$\text{Relative survival rate} = \frac{\text{Cumulative survival rate}}{\text{Expected survival rate}}$$

The history of our experience in the treatment of maxillary cancer can be divided into four chronological periods the periods of preoperative irradiation (1957–66) intraarterial infusion of radiosensitizers (1967–69) Linac X ray irradiation (1970–71) and 5 FU intraarterial infusion (1972–73) Randomized studies have been carried out since 1967 The crude survival rate for each period is shown in Table IV and the relative survival rate in Fig 2 The treatment policy of each period will be detailed in the following

From the outset we have chosen radiotherapy as the primary mode of treatment We gave 4–5 000 rad over 4–5 weeks with 200 kVp X ray or 6–7 000 rad over 6–7 weeks with Co-60 γ ray from 1957 through 1966 A maxillectomy was done whenever indicated 6–8 weeks after the completion of radiotherapy The 5 year relative survival rate for 282 cases treated during this period was 22 8%

Those 282 cases consisted of a group of 114 cases which received a combined radiotherapy and surgery and of another group of 168 cases which received radiotherapy only The 5 year relative survival rates for these two groups

Table IV. *Crude survival rates for maxillary sinus carcinoma according to different treatment policies (statistics as of January 1975)*

	1 year	2 years	3 years	4 years	5 years
1957–66 period of preoperative irrad	162/282 585	99/282 351	73/282 259	62/282 220	52/282 184
1967–69 period of various drugs i a i [a]	91/130 700	56/130 431	49/130 377	44/130 338	39/130 300
1970–71, period of Linac X ray irrad [b]	34/61 557	15/61 246	12/61 197	9/61 148	4/30 133
1972–73 period of 5 FU i a i [a]	61/80 763	40/77 519	12/34 353		
Total	354/561 636	198/535 370	140/491 285	113/463 244	92/419 220

[a] i a i intra arterial infusion
[b] Statistics as of July 1975

were 36 9 and 12 7%, respectively As shown in Fig 3, the relative survival rate was higher in the former than in the latter in both Stages I and II It is obvious from these data that irradiation alone is not an adequate treatment, particularly for advanced cases

Control studies were performed from 1967 through 1969 using different kinds of radiosensitizers (5-FU, Cyclohexanol succinate, Ametohepazone, and Bleomycin) (Shigematsu, 1971) The fifth group, which received radiotherapy alone, served as the control group An [illegible] was performed at the beginning of therapy to facilitate drainage and continuous observation of the sinus cavity A maxillectomy was performed in recurrent cases only The difference in the 3-year survival rate between the 5-FU group and other groups was statistically significant at the level of 1%, i e the 3-year relative survival rates for 5 FU group, other radiosensitizer groups and radiotherapy group were 56, 40 and 38%, respectively However, because of intercurrent diseases, their 5-year relative survival rates approached one another closely The relative survival rates for these three groups are shown in Fig 4 The 2 year local non recurrence rates for the above three groups were 40, 22, and 14% respectively As shown in Table V, the 2-year local control rate for the 5 FU group was significantly better than those for other groups

The combination of radiotherapy and hyperbaric oxygen therapy was tried from 1970 to 1971 However, no difference was noted in the survival rates between the group treated

Table V *Local control rates according to different primary therapies*

	No of cases	Clearance rate		Non recurrence rate			
		clinical	microsc	0 5 y	1 y	1 5 y	2 y
1967–69							
5 FU i a i [a] w Co irrad	25	760	792	600	480	458	401
i a i of other drugs w Co irrad	35	600	543	371	242	219	219
Co irrad alone w/o i a i	45	489	429	289	163	140	140
1972–73							
5 FU i a i Co irrad 5000 rad	23	565	783	739	545	476	429
5 FU i a i Co irrad 3 500 rad	26	577	730	640	542	522	500
5 FU i a i Co irrad w currettage	25	520	800	760	652	619	600
5 FU i a i Co irrad w/o currettage	24	625	708	609	435	391	348

[a] i a i intra arterial infusion

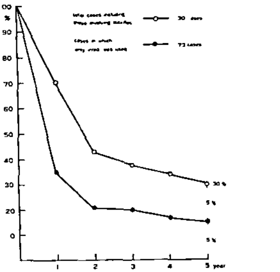


Fig 5 Crude survival rates (1967–69) The area between the two lines indicates the salvage effect of maxillectomy

under ×3 atmospheric pressure and the control group treated under normal atmospheric pressure The survival rate in 61 cases treated during this period was also worse than expected, as shown in Table IV and Fig 2

Since 1972 we have again used 5-FU intra-arterial infusion combined with irradiation 5 000 rad was given in 10 fractions over 5 weeks in one group and 3 500 rad in 7 fractions over 3 5 weeks in another group These doses correspond to 7 000 rad and 5 000 rad in daily pace, respectively The previous policy of performing an antrostomy for recurrent cases only has not been changed Changes in fraction dosage of radiation, trial of radiosensitizers such as BUdR and curettage of maxillary sinus were made as needed The 3-year relative survival rate for 80 cases treated by the end of 1973 was 44 %, as shown in Fig 2 The 2 year local non recurrence rates for 5 000 rad and 3 500 rad groups were 43 and 50 %, respectively, which was not a statistically significant difference The same local non recurrence rates for curettage and non curettage groups were 60 and 35 % respectively which was significantly different, as shown in Table V

The policy of performing a maxillectomy in recurrent cases only enabled us to evaluate the therapeutic value of maxillectomy The contribution of maxillectomy was estimated in the just half of the 30 % 5 year crude survival rate in all cases treated during 1967–1969 On the other hand, the other half of the 5-year crude survival rate drove from the primary therapy, as shown in Fig 5

The relation of the local control rates to the degrees of clinical effect of the primary therapy was studied in the cases treated between 1967 and 1973 As shown in Fig 6, the 2 year local control rates for Degrees 1 and 2 and Degree 3 were as low as 3 and 18 %, respectively It is obvious from this figure that a maxillectomy is indicated when the initial radiotherapy with chemotherapy has given Degree 1, 2 or 3 in the criteria of clinical effect

In conclusion, the best mode of treatment in our experience is the combination of Co-60 γ-ray irradiation (5 000 rad/25 fr/5 wk, 2 portals with wedge pair filter) and continuous intra-arterial infusion of 5-FU (ca 1 500 mg in amount) An antrostomy is required at the beginning of the treatment and a curettage of the sinus may be needed A maxillectomy must be performed in irradiation failure cases Intra-

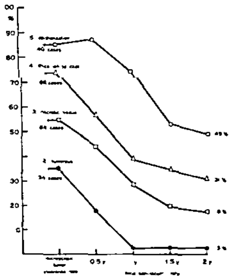


Fig 6 Local control rates according to the degrees of clinical effect of primary therapy (1967–73)

Table VI. *Clinical studies on malignant tumors of the nose and paranasal sinuses from the literature sources*

Reporter	Institute	Year of report	Period of treatment	Primary site and histology	Treatment or comment	3 year crude survival rate cases	%	5 year crude survival rate cases	%
Öhngren	Radium hemmet Stockholm	1933		Malig tumor of nose & para nasal sinuses	SG→RT			72/187	39
Leroux Bobert	F Curie	1951	1935–50	Malig tumor of nose & para nasal sinuses	SG RT	27/71	38	14/45	31
Baclesse	F Curie	1952	1929–45	Malig tumor of nose & para nasal sinuses	RT			16/55	29
Larsson	Radium hemmet Stockholm	1954	1940–47	Carcinoma of nose & para nasal sinuses	All cases			68/256	27
					RT→SG			51/114	45
					RT			17/142	12
Figi	Mayo C	1958	1935–48	Malig tumor of nose & para nasal sinuses	SG→RT	159/381	42	127/381	33
Cocchi	Zurich	1960	1919–58	Malig tumor of nose & para nasal sinuses except maxill sinus	RT SG	10/54	19	7/50	14
Zange	Jena	1963	1931–55	Malig tumor of nose & para nasal sinuses	All cases SG RT			98/277	35
				Carcinoma of paranasal sinus	Primary			40/133	30
				Sarcoma of paranasal sinus	Primary			14/35	40
				Carcinoma of nasal cavity	Primary			19/30	63
				Sarcoma of nasal cavity	Primary			7/23	30
				Malig tumor of nose & para nasal sinuses	Secondary			18/56	32
Wustrow	Koln	1963	1955–63	Malig tumor of nose & para nasal sinuses	SG→RT	53/96	55	33/96	35
				Carcinoma of nose and para nasal sinuses	SG→RT	33/61	54	19/61	31
				Sarcoma of nose and para nasal sinuses	SG→RT	18/31	58	12/61	39
Frazell	Memorial H	1963	1935–54	Carcinoma of nose and para nasal sinuses	All cases			103/369	28
				Carcinoma of nose and para nasal sinuses	SG			89/253	35
				Carcinoma of nose and para nasal sinuses	RT			14/98	14
Salem	Lima	1963	1952–61	Carcinoma of paranasal sinus	SG→RT			27/76	33

Table VI (*cont.*)

Reporter	Institute	Year of report	Period of treatment	Primary site and histology	Treatment or comment	3 year crude survival rate		5-year crude survival rate	
						cases	%	cases	%
Spratt	Ellis Fish Ca. H.	1965	1940–64	Carcinoma of maxillary sinus	SG RT			10/69	15
Sisson	Upstate Med. Center	1965	1951–61	Carcinoma of maxillary sinus	SG, RT			12/54	22
				Carcinoma of maxillary sinus	RT→SG			9/32	28
Macbeth	Oxford	1965	1951–59	Carcinoma of paranasal sinus	SG RT			15/40	38
				Carcinoma of paranasal sinus	Compl. tr.			15/34	44
Mårtensson	Karolinska Stockholm	1965	1940–59	Carcinoma of nose and para nasal sinuses	All cases			148/513	29
				Carcinoma of nose and para nasal sinuses	RT→SG			87/195	45
				Carcinoma of nose and para nasal sinuses	RT			61/318	19
Ireland	Tront	1966	1951–63	Malig. tumor of paranasal sinus	SG RT	32/100	32	18/81	22
Boone	M. C. Anderson Hospital	1968	1954–63	Malig. tumor of nose and para nasal sinuses	SG RT			51/121	43
Badib	Roswell Park	1969	1925–65	Malig. tumor of paranasal sinus	SG RT			44/304	14
Badib	Roswell Park	1969	1942–64	Malig. tumor of nasal cavity	SG RT			32/57	57
Gallagher	Michigan	1970	1955–64	Carcinoma of maxillary sinus	SG RT			15/49	31
Lederman	Royal Marsden	1970	1933–67	Carcinoma of maxillary sinus	All cases	76/236	32	49/207	24
				Carcinoma of maxillary sinus	SG→RT	25/56	47	19/55	35
				Carcinoma of maxillary sinus	RT→SG	42/113	35	26/99	26
				Carcinoma of maxillary sinus	RT	9/67	13	4/53	08
				Malig. tumor of maxillary sinus	All cases	99/282	35	62/248	25
				Malig. tumor of ethmoid cells	All cases	44/128	34	27/115	24
				Malig. tumor of nasal cavity	All cases	65/113	60	37/97	38
Tabb	Tulane	1971	1958–68	Malig. tumor of maxillary sinus	SG RT			28/108	27
Hussel	Innsbruck	1971	1953–70	Malig. tumor of paranasal sinus	SG RT	13/60	21	11/60	18
Lewis	Memorial Hospital	1972	1935–65	Malig. tumor of nose and para nasal sinuses	All cases			191/677	28
				Malig. tumor of nose and para nasal sinuses	SG→(RT)			168/530	32
				Malig. tumor of nose and para nasal sinuses	RT→(SG)			23/144	16

Table VI (cont)

Reporter	Institute	Year of report	Period of treat ment	Primary site and histology	Treatment or comment	3 year crude survival rate		5 year crude survival rate	
						cases	%	cases	%
Torre	François Baclesse	1973	1954–70	Carcinoma of maxillary sinus	RT→SG			17/83	20
Yamashita	Cancer I Japan	1968	1959–63	Carcinoma of maxillary sinus	RT→SG	34/107	32	23/107	21
Miyake	Keio Japan	1971	1957–68	Carcinoma of maxillary sinus	SG RT (CH)	41/115	36	23/79	26
Sato	Tokyo Japan	1972	1962–72	Carcinoma of maxillary sinus	RT+CH			89/116	77
Matsuura	Cancer C Japan	1972	1962–66	Carcinoma of maxillary sinus	RT→SG	46/90	51	38/90	42
Shimizu	Kobe Japan	1974	1959–72	Carcinoma of maxillary sinus	SG RT	95/230	41	80/210	38
Saito	Kyoto Japan	1974	1955–72	Carcinoma of maxillary sinus	RT+(CH) →SG	47/159	30	37/137	27
Sakai (in this paper)	Osaka Japan	1975	1957–73	Carcinoma of maxillary sinus	RT+(CH) →(SG)	140/491	29	92/419	22

cavitary irradiation may be used for small re current tumors A radical neck dissection is necessary for cervical metastasis

Representative studies in this field were shown in Table VI The long term survival rate in maxillary cancer is still disappointing There is an urgent need for comprehensive studies to establish the optimal combination of radiotherapy chemotherapy and surgery

ACKNOWLEDGEMENT

The authors wish to express their thanks to Prof C A Hamberger (Sweden) Prof G Mårtensson (Sweden) Prof F Wustrow (W Germany) Prof T Naito and Prof Y Shigematzu for their helpful suggestions and advice The authors would like to pay tribute to all the members of our study group who have contributed so much during the past 18 years of existence

ZUSAMMENFASSUNG

Die von 1957 bis 1974 behandelten 908 Falle von malignen Tumoren der Nase und der Nasennebenhohlen wurden statistisch analysiert Die Absolute und Relative Uber lebensraten wurden im Januar 1975 je nach der Behand lungsmethode bzw je nach dem klinischen Stadium er rechnet Die Stadium Gruppierung des Kieferhohlen Karzinoms auf Grund der TNM Klassifikation wurde durch den Vergleich der Uberlebensrate vorgeschlagen

Als die erste Behandlungsstufe des Kieferhöhlen Karzi noms ist eine Bestrahlung mit Co-60 Gammastrahlen 5000 rad wahrend 5 Wochen kombiniert mit einer intraarteriel *len Dauerinfusion von 5 FU ca 1500 mg als Gesamt* dosis am besten Eine Antrostomie nach Caldwell Luc wird am Anfang der Behandlung durchgefuhrt und eine *Auskratzung der Kieferhohle wird wahrend der Bestrah* lung empfohlen Nur auf ein Rezidiv beschrankt soll eine Maxillektomie durchgefuhrt werden

REFERENCES

Badib A O 1969 Treatment of cancer of nasal cavity *Am J Roentgenol 106* 824

— 1969 Treatment of cancer of the paranasal sinuses *Cancer 23* 533

Baclesse F 1952 Les cancers du sinus maxill de l eth moide et des fosses nasales *Ann Otolaryngol* (Paris) 69 465

Boone M L M 1968 Malignant disease of the para nasal sinuses and nasal cavity *Am J Roentgenol 102* 627

Cocchi U 1960 Die Strahlentherapie der malignen Tu moren der inneren Nase und der Nasennebenhohlen (ohne Oberkieferhohlen) *Oncologie 13* 370

Cutler S J 1958 Maximum utilization of the life table method in analyzing survival *J Chron Dis 8* 699

Ederer P 1961 Relative survival ratio A statistical methodology *Nat Cancer Inst 6* 101

Figi F A 1958 *The treatment of cancers of the nose and* accessory sinuses treatment of cancer and allied dis eases In *Tumor of the Head and Neck* vol III p 279 Paul B Hoeber

Frazell E L 1963 Cancer of the nasal cavity and accessory sinuses Report of the management of 416 patients *Cancer 16* 1293

Gallagher T M 1970 Treatment of malignancies of paranasal sinuses *Laryngoscope 80* 924

Hussel B 1971 Behandlungsergebnisse bei malignen Neben Hohlen tumoren in Zeitraum von 1953 bis 1970 *Monatsschr Ohrenheilkd 105* 558

Ireland P E 1966 Carcinoma of accessory nasal sinuses *Ann Otol Rhinol Laryngol 75* 698

Larsson L G & Mårtensson G 1954 Carcinoma of the paranasal sinuses and the nasal cavities *Acta Radiol* (Stockh) *42* 147

Lederman M 1970 Tumours of the upper jaw Natural history and treatment *J Laryngol Rhinol Otol 84* 369

Leroux Robert J 1951 Cancer du massif ethmoido-maxillaire *Ann Otolaryngol* (Paris) *68* 617

Lewis J S 1972 Cancer of the nasal cavity and para nasal sinuses *J Laryngol Otol 86* 255

Macbeth R 1965 Malignant disease of the paranasal sinuses *J Laryngol Otol 79* 592

Mårtensson G 1965 Carcinoma of the paranasal sinuses and the nasal cavities *Exc Med Intern Congress Series 113*

Öhngren G 1933 Malignant tumours of the maxillo-ethmoidal region *Acta Otolaryngol* (Stockh) Suppl *19*

Sakai S 1974 *Maxillary Cancer* Kanehara KK Tokyo

— 1967 Proposal for the classification of carcinoma of paranasal sinuses *Acta Otolaryngol* (Stockh) *63* 42

— 1971 Proposal for the TNM classification and its extended application for malignant tumors of head and neck regions *Acta Otolaryngol* (Stockh) *72* 370

— 1972 Diagnosis and TNM Classification of maxillary sinus carcinoma *Acta Otolaryngol* (Stockh) *74* 123

Salem L E 1963 Carcinoma of the paranasal sinuses *Am J Surgery 106* 876

Sato Y 1970 Combined surgery radiotherapy and regional chemotherapy in carcinoma of paranasal sinuses *Cancer 25* 571

Schultz M D 1969 Clinical staging of cancer and end result reporting *Cancer J for Clinicians 19* 158

Schwab W 1968 Zur Klassifizierung und Dokumentation bösartiger Geschwulste in Hals Nase-Ohren Bereich unter Berucksichtigung der Richtlinien der UICC (TNM System) *J Laryngol Otol 47* 21

Shigematsu S 1971 Recent trials in the treatment of maxillary sinus carcinoma with special reference to the chemical potentiation of radiation therapy *Acta Otolaryngol* (Stockh) *71* 63

Sisson G A 1963 Cancer of the maxillary sinus Clinical classification and management *Ann Otol Rhinol Laryngol 72* 1050

Spratt T S 1965 Therapy and staging in advanced cancer of the maxillary antrum *Am J Surgery 111* 502

Tabb H G 1971 Cancer of the maxillary sinus An analysis of 108 cases *Laryngoscope 81* 818

Torre P 1973 Le traitement radio-chirurgical des cancers du sinus maxillaire *Ann Otolaryngol* (Paris) *90* 353

UICC 1974 TNM General Rules Geneva

Wustrow F 1968 Vorschlage zur Klassifizierung der Tumoren in Bereich der Nase und ihrer Nebenhohlen nach dem TNM System *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 191* 640

— 1965 *Die Tumoren des Gesichts schadels* pp 366–367 Urban & Schwarzenberg

Zange J 1963 25 Jahre Behandlung bösartiger Geschwulste der Nase und Nebenhohlen in Jena und ihr Ergebnis *Z Laryngol Rhinol 42* 613

S Sakai M D
3 11-4 Aoyama dai
Suita shi
Osaka fu
Japan

Sinuiter

— It should be noted that ampicillin, like phenoxymethylpenicillin, does not penetrate into sinus secretions from blood in reliable concentrations. —

H Gnarpe and C. Lundberg, Scand J. Inf Dis 3 257, 1971

*

— Our investigation shows that doxycycline passes into the sinus secretions even under severe inflammatory conditions. —

C. Lundberg et al, Lancet II 107, 1968

*

ibramycin®

doxycyklin

en dos per dag

Pfizer

Pfizer AB, Taby

TRANSACTIONS OF THE XIXTH CONGRESS OF THE SCANDINAVIAN OTO-LARYNGOLOGICAL SOCIETY

REYKJAVIK, ISLAND, JUNE 26–28, 1975

Acta Otolaryngol 82 185–192, 1976

IMMUNODEFICIENCY SYNDROMES WITH OTORHINOLARYNGOLOGICAL MANIFESTATIONS

P Berdal, P Brandtzæg, S S Frøland, S D Henriksen and S Skrede

From the Department of Otolaryngology Institute of Pathology Institute of Immunology and Rheumatology Institute of Bacteriology and Institute of Clinical Biochemistry Rikshospitalet University of Oslo Oslo Norway

Abstract Among patients with recurrent protracted or chronic infections of the respiratory tract involving the middle ear 18 were found to have immunodeficiencies In 10 of the patients deficiency of immunoglobulins be longing to the IgG IgA and IgM classes was found Seven patients had an isolated IgA deficiency One patient had a combined immunodeficiency with defects of the T-cell system and the B cell system One patient had an isolated T-cell deficiency

Immunodeficiency may be the reason for fail ure of treatment in patients with recurrent or chronic infections of the respiratory tract and the middle ear Among patients suffering from such diseases admitted to the Department of Otorhinolaryngology, Rikshospitalet, Oslo in the period 1960–1974 18 were found to have immunodeficiency

The types of deficiency may be grouped as follows

	No of cases
Generalized hypogammaglobulinaemia (IgG IgA IgM)	9
T-cell defect	1
Combined defect of B and T-cell systems	1
Selective IgA deficiency	7

The functions of the B cell system were examined by quantitation of serum immunoglobulins belonging to the IgG, IgA and IgM classes and by immunofluorescence microscopy of the nasal mucosa, and by B-lymphocyte determination in blood and occasionally also in lymphoid tissue suspensions from the tonsils The T cell functions were examined *in vitro* by testing blastoid transformation with various antigens and mitogens, and *in vivo* by testing delayed hypersensitivity

Table I shows relevant findings in the 9 cases with generalized hypogammaglobulinaemia and in the patient (♀ H S) with combined B- and T-cell deficiency The serum concentrations of the immunoglobulins refer to the date of diagnosis Normal adult values in this laboratory are for IgG, 750–1 500 mg/100 ml, for IgA, 50–350 mg/100 ml, and for IgM, 35–200 mg/100 ml The two figures given for leukocytes, for percentages of lymphocytes and neutrophils and for sedimentation rate indicate the observed range during the complete observation period The blood cell counts did not show any remarkable deviations from normal values Noticeable were however, the sedimentation rates which in all cases were unexpectedly low A number of "acute-phase proteins" (α_1-antitrypsin, orosomucoid, haptoglobin, fibrinogen C reactive protein and complement C_3) were also determined Only small changes were found, and in most cases an "acute phase reaction", which normally accompanies acute bacterial or viral infections, was absent

The predominant pathogenic organism, *Haemophilus influenzae (H infl)* could be cultivated from the nasal/nasopharyngeal se-

Table I *Generalized hypogammaglobulinaemia (IgG IgA IgM) in recurrent/chronic rhinitis sinusitis bronchitis recurrent/chronic otitis*

D-day date for diagnosis
PNC pneumococci
ND non detectable

Sex Initials Birth date	Age D day 1975	Serum Ig mg/100 ml IgG	IgA	IgM	Leuko- cytes	Lympho- cytes (%)	Granulo- cytes (%)	SR	Patho- genic microbes	B lympho cytes in blood[a]	T lymphocyte functions in vitro[b]	Immunohistochemistry nasal mucosa
♂ E L 26 10 50	3/24	50	ND	ND	4 800 16 400	50 16	50 84	3 3	*H Infl*	<0 1%	Normal	Tissue Ig ND Local synthesis Ig markedly decreased
♀ S H 18 05 50	15/24	250	15	ND	6 000 11 900	26 29	74 71	7 10	*H Infl*	<0 1%	Normal	Tissue Ig ND Local synthesis Ig ND SC synthesis normal
♂ G S 25 08 62	5/12	ND	ND	ND	7 500 17 300	54 50	46 50	10 10	*H Infl*	<0 1%	Normal	Tissue Ig ND Local synthesis Ig ND SC synthesis normal
♂ O S S 06 01 66	2 8	<20	ND	13	8 100 8 300	62 67	38 33	3 17	*H Infl*	<0 1%	Normal	
♂ G U L 01 12 64	4/10	150	17	10	8 500 15 700	59 24	41 76	8 16	*H Infl*	<0 1%	Normal	Tissue IgG decreased IgA and IgM ND Local synthesis Ig ND SC synthesis normal
♂ Ø M 11 10 51	7/24	130	ND	ND	5 100 11 600	22 28	78 72	3 3	*H Infl*	<0 1%	Normal	Tissue Ig negl gible Local synthesis Ig ND SC synthesis normal
♂ K J L 12 11 64	5/8	60	ND	20	7 500 10 000	20 53	80 47	4 5	*H Infl* *(PNC)*	<0 1%	Normal	Tissue IgA and IgG ND IgM greatly decreased local synthesis Ig greatly decreased SC synthesis normal
♂ S A L 28 05 66	6/8	150	ND	30	4 700 6 500	43 45	57 55	22 3	*H Infl* *(PNC)*	<0 1%	Normal	
♂ P S 16 03 37	35/38	20	20	20	7 700 14 400	20 53	80 47	5 11	*H Infl*	15%	Normal	Tissue Ig ND Local synthesis Ig ND SC synthesis normal
♀ H S 04 04 28	32/47	ND	ND	ND	4 100 12 300	50 16	50 84	5 10	*H Infl*	<0 1%	Impaired	

[a] As identified with immunofluorescence technique Range in normals 3–22%
[b] Stimulation with the following mitogens PHA PWM ConA PPD MLC (mixed lymphocyte culture)

cretions in all cases and in some cases depending on the clinical state also from middle ear secretions and bronchial secretions

The numbers of B cells in blood as shown by surface immunoglobulin immunofluorescence were below the lowest normal range except in case 9 (♂ P S) in which the B-cells comprised 15% of circulating lymphocytes In this patient the maturation to plasma cells was apparently blocked The T cell lymphocyte functions were normal in all patients except in case 10 (♀ H S) in which it was slightly impaired The immunohistochemistry of the nasal mucosa in all cases examined showed a uniform picture characterized by nondetectable or markedly decreased local synthesis of immunoglobulins and also by non detectable or greatly decreased amounts of serum derived immunoglobulins in the mucosal tissue The epithelial synthesis of secretory component (SC) was normal in all cases

Table II shows the fundamentals of the treatment in the patients with generalized hypogammaglobulinaemia Substitution therapy with gammaglobulins was given in most cases (7 patients out of 10) This treatment was not satisfactory as the symptoms and the *H infl* infection persisted Elimination of the *H infl* infection appears to be essential for an adequate clinical response However we did not succeed in this by a systematic substitution therapy alone Four patients had arthritis of rheumatoid type In three of these the arthritis developed during substitution therapy In two cases synovectomy was performed The synovitis was of a proliferative exudative nondestructive type Having tried supplementary treatment with penicillin ampicillin and sulphonamides without success we have since 1971 combined the substitution therapy with trimethoprim sulphamethoxazol This remedy has been found to be therapeutically decisive The *H infl* infection has disappeared and the symptoms have subsided

In 3 patients the trimethoprim sulphamethoxazol therapy alone proved sufficient for complete symptomatic relief and disappearance of *H infl* from the secretions Side effects of the trimethoprim sulphamethoxazol treatment were not observed during the period of study which is now almost 4 years The general condition of these patients improved substantially and they are all able to lead a normal life

In Table III fundamental features from the patient with T cell deficiency are shown The girl still suffers from pulmonary candidadiasis Treatment with transfer factor has not been convincing with regard to immunological competency and lung changes However her general condition has improved Trimethoprim sulphamethoxazol has exerted a favourable effect on the mucosal infections *H infl* has disappeared Statomotor and mental development is fair but lung function is poor However she is able to attend school

Table IV presents a survey of the patients with selective IgA deficiency The clinical manifestations include allergic rhinitis nasal polypi bronchial asthma chronic sinusitis chronic otitis and hyperplasia of the tonsils Serum IgA was non detectable or markedly decreased Immunohistochemistry showed a corresponding mucosal IgA deficiency whereas local synthesis of IgG and IgM was normal or increased Epithelial SC was normal in all cases examined Three of our patients are epileptics and have been treated with phenytoin This drug is considered to be of aetiological importance in some instances of IgA deficiency

Substitution therapy is probably of no value in IgA-deficient patients even when available IgA preparations are used These consist mainly of monomeric (7S) IgA which is not transported to the mucosal surfaces like secretory IgA An efficient surface protect on therefore cannot be restored

Table V summarizes features and treatment of patients with generalized hypogammaglobulinaemia (Bruton type and other B lymphocyte deficiencies)

Table VI shows a sim[illegible] selective IgA deficiencies

Table II *Generalized hypogammaglobulinaemia (IgG, IgA, IgM) substitution treatment ch*
therapy
PNC=Pneumococci

Sex Initials Birth date	Age D-day 1975	Serum Ig mg/100 ml Date	IgG	IgA	IgM	Gamma globulin[a]
♂ E L 26 10 50	3/24	1962	120	ND	ND	12% 30 ml/2 week
		Febr 1972	350	ND	20	since 1968 16½%
		June 1972	270	ND	ND	10 ml/2 weeks
		Aug 1972	120	ND	ND	
♀ S H 18 05 50	15/24	Jan 1972	250	15	ND	1965–1971 10 ml/2 weeks
		March 1973	ND	ND	ND	No substitution
♂ G S 25 08 62	5/12	April 1967	ND	ND	traces	
		April 1969	150	ND	ND	5 ml/2 weeks
		Oct 1971	40	ND	ND	5 ml/2 weeks
		July 1972	20	10	30	5 ml/2 weeks
♂ O S S 06 01 66	2/8	April 1967	ND	ND	ND	No substitution
		April 1968	30	30	20	No substitution
		April 1970	10	16	17	5 ml/2 weeks
		Oct 1971	220	ND	10	5 ml/2 weeks
						March 1973 10 ml/? weeks
♂ G U L 02 12 64	4/10	June 1969	150	17	10	May 1969 5 ml/2 weeks
		Dec 1971	200	ND	35	
		Dec 1972	50	ND	ND	March 1973 10 ml/2 weeks
♂ Ø M 11 10 51	7/24	Oct 1968	ND	ND	ND	(12%/2 weeks)
		Oct 1969	150	13	16	10 ml/2 weeks
		Dec 1970	250	2	ND	10 ml/2 weeks
		Aug 1971	220	ND	90	10 ml/2 weeks
		Febr 1973	160	ND	ND	10 ml/2 weeks
♂ K J L 12 11 64	5/8	Oct 1972	60	ND	20	No substitution
		Febr 1973	30	ND	ND	No substitution
♂ S A L 28 05 66	6/8	Sept 1972	150	ND	30	No substitution
		Febr 1973	40	ND	ND	No substitution
♂ P S 16 03 37	35/38	Jan 1972	120	10	30	10 ml/4 weeks
		Febr 1972	20	20	20	
						Since Jan 1974 20 ml/4 weeks
♀ H S 04 04 28	32/47	1960	ND	ND	ND	
		1972	250	ND	ND	20 ml/2 weeks

[a] Usually gammaglobulin (Kabi) 16.5%
[b] Normal values 5–17 U/l
[c] Normal values 4–17 U/l
[d] Normal values 75–160 U/l

Trimethoprim (T) Sulphamethoxazol (S) Daily dosage	Clinical response	Bacteriol effect	Transaminases U/l		
			GOT[b]	GPT[c]	LDH[d]
T 160 mg } S 800 mg } 2 since Oct 1971	*Good*	*H Infl* eliminated	*Nov 1971* 10	10	133
			Aug 1972 9	11	127
T 160 mg } S 800 mg } 2 since Dec 1971	Good	*H Infl* eliminated	Jan 1972 6	6	
			March 1973 6	4	
T 40 mg } S 200 mg } 2 since Oct 1971	Good	*H Infl* eliminated			
T 40 mg } S 200 mg } 2 since Oct 1971	Good	*H Infl* eliminated			
T 40 mg } S 200 mg } 2 since Dec 1971	Good	*H Infl* eliminated	Jan 1972 13	7	210
			March 1973 10	5	
	Fair				
	Fair				
T 160 mg } S 800 mg } 2	Fair				
	Fair	*H Infl*	Jan 1973		
since Febr 1973	Good	eliminated	12	10	
T 40 mg } S 200 mg } 2	Good	*H Infl* eliminated	March 1973 11	10	170
(Penicillin 1 M u)	Good	*PNC* eliminated			
T 40 mg } S 200 mg } 2 since June 1975		New infect *H Infl*			
T 160 mg } S 800 mg } 2 Jan –June 1972	Good	*H Infl* eliminated	Febr 1972 7	8	
			March 1973 10	9	
T 160 mg } S 800 mg } 2 since July 1971	Good	*H Infl* eliminated			

Table III *T-cell deficiency Recurrent/chronic otitis mastoiditis Septicemia, osteomyelitis pulmonary candidadiasis*

Sex Initials Birthday	Age D-day 1975	Serum Ig mg/100 ml IgG	IgA	IgM	Leuko-cytes	Lympho-cytes	SR	Bone marrow	Pathogenic microbes
♀ B V R 31 12 67	3/7	5 000	80	150	18 100 44 000	62 75	127	Increase in lymphocytes and plasma cells	*H Infl* in blood synovial fluid middle ear secretion *Candida alb* bronchial secretion

[a] As identified with immunofluorescence technique Range in normals 3–22%
[b] Stimulation with the following mitogens PHA PWM ConA PPD MLC (mixed lymphocyte culture)

Table IV *Selective IgA deficiency*

Sex Initials Birthday	Age D-day 1975	Clinical manifestations	Serum Ig mg/100 ml Date	IgG	IgA	IgM	Leuko-cytes	Lympho-cytes (%)	SR	Pathogenic microbes
♂ B L 02 08 14	53/61	Allergic rhinitis polyps	09 01 73	1 950	ND	230	5 600 5 900	34 49	78 28	*PNC* nasal secr
♂ P H 14 09 63	14/21	Spleen rupture April 1967 resp infect some months later	29 03 69 20 01 72	1 750 1 850	ND 30	109 120	8 700 11 900	39 38	3 10	*Yellow staphc* nasal secr
♂ K B 18 05 33	39/42	Chron otitis 1960 tymp plasty granulation tissue otorrhea.	09 0? 72	2 100	ND	50	9 700 11 000		38 3	*Proteus pseudomonas* ear secr
♂ B B 10 11 67	5/7	Chron otitis mastoidect bil 2 years old Polyp right ear tymp plasty	06 02 73	2 200	ND	100	7 300	47	8	Not examin
♀ R A K 22 03 47	26/27	Chron purulent sinusitis Epilepsy[a]	04 09 74	700	ND	130	8 ?00		4	*H Infl PNC meningoc*
♂ J P W 04 12 45	28/28	Nasal polypi br asthma Epilepsy[a]	15 02 74 10 09 74	2 200 2 200	40 20	90 80	5 ?00 6 900	33 26	4 2	*Yellow staphc* nasal secr
♂ T P 25 03 70	5/5	Hyperplasia tonsils Epilepsy[a]	31 01 75 04 03 75	1 200 1 050	ND ND	190 220	4 300 11 100	43 34	11 7	None patho *PNC* nas secr

[a] Phenytoin [illegible] on

lympho-ytes in lood[a]	T lymphocyte functions in vitro[b]
%	PHA response slightly impaired PWM response greatly impaired ConA response greatly impaired MLC good response PPD no response

listology	Immunohistochemistry
olyps lymphoc plasmac eosinoph	Nasal mucosa IgA ND local synthesis IgG increased local synthesis IgM normal SC synthesis normal
	Not examined
Aastoid squam ep lymphoc plasmac eosinoph	Nasal mucosa IgA ND local synthesis IgG and IgM normal SC synthesis normal mastoid IgA ND dense infiltration IgG producing cells cuboid ep containing SC
Aastoid cuboid ep lymphoc plasmac	Nasal mucosa IgA negligible IgG and IgM normal local synthesis IgA slightly reduced IgG and IgM normal SC synthesis normal
	Not examined
'olyps lymphoc plasmac macroph mastc eosinoph	Nasal mucosa IgA decreased IgM IgG and SC normal
Tonsils lymphoid tissue well developed	Nasal mucosa IgA markedly decreased IgM and IgG local synthesis increased SC synthesis normal Tonsils IgG and IgM cells normal number Immature IgA cells in some follicles

Table V *Generalized hypogammaglobulinaemia (IgG, IgA, IgM) Main features*

Bacterial inflammations air passages middle ears mainly caused by *haemophilus influenzae*
Blood survey
- Leukocytes lymphocytes granulocytes normal
- Haemoglobin normal
- Serum iron lowered
- SR low

Serum proteins
- Total protein lowered
- Gammaglobulin lowered

Lymphocyte populations in blood
- B lymphocytes missing
- T lymphocytes normal functions

Nasal mucosa
- In tissue Ig greatly reduced/missing
- Synthesis Ig greatly reduced/missing
- Synthesis SC normal

Treatment

Substitution therapy
- Gammaglobulins every 2 weeks

Chemotherapy
- Trimethoprim-sulphamethoxazol
- (*Haemophilus influen ae*) penicillin (pneumococci)

Supporting treatment
- Iron
- Physiotherapy
- Local treatment of otitis

Table VI *Selective IgA deficiency Main features*

Bacterial inflammations
- air passages middle ears

Blood survey
- Leukocytes lymphocytes granulocytes normal
- Haemoglobin normal
- Serum iron seldom lowered
- SR low

Serum proteins
- Total protein not lowered
- Total gammaglobulins not lowered

Nasal mucosa
- Synthesis IgA greatly reduced/missing
- Local synthesis IgG IgM normal/increased
- Synthesis SC normal

Treatment

Substitution therapy?
Chemotherapy in case of clinical manifestations (NB bacteriology)
Supporting treatment
Local treatment/surgical treatment of otitis
Phenytoin medication stop

Table VII *Diagnosis of immunodeficiencies*

Clinically
Recurrent/chronic rhinitis sinusitis bronchitis
Recurrent/chronic otitis
Recurrent pneumonia
[illegible]
[illegible]
[illegible]
Electrophoresis of serum proteins
Quantitation of immunoglobulin classes in serum
Lymphocyte examinations
B lymphocytes quantitation
T lymphocytes quantitation and functional in vitro studies with mitogens
Immunohistochemistry of nasal mucosa

In Table VII the most important diagnostic features of immunodeficiencies are compiled

Immunodeficiencies may be involved in diseases causing a variety of clinical symptoms, and the affected patients may turn up in different hospital departments and at different ages We do not know the actual frequency of such patients among those being attended by the otolaryngologist However, there is no doubt that most individuals with immunodeficiency have had infections of their upper respiratory tract in early childhood Our report should serve to alert the otolaryngologist to these problems, and be helpful in the diagnosis

ZUSAMMENFASSUNG

Unter den Patienten die in den Jahren 1960–1974 wegen rezidivierender langanhaltender oder chronischer Infektionen der Luftwege mit impliziertem Mittelohr an der Hals Nasen Ohrenklinik Rikshospitalet Oslo behandelt wurden hatten 18 Patienten Immundefekte Bei 10 Patienten wurde ein Immunglobulinmangel in der IgG IgA und IgM Klasse gefunden Sieben Patienten hatten einen isolierten IgA Mangel Ein Patient hatte einen kombinierten Immundefekt des T Zellen und B Zellen Systems Eine Patientin hatte isolierten T Zellenmangel

Acta Otolaryngol 82 193–195, 1976

IMMUNOLOGICAL DEFECTS IN CHILDREN WITH CHRONIC OTITIS MEDIA

P Karma, A Palva and E Kokko

From the Department of Otolaryngology University of Oulu Oulu Finland

Abstract A total of 59 children with chronic otitis media were examined for possible immunological defects by determination of serum gammaglobulin and immunoglobulins IgG IgA and IgM and by a tuberculin test and additionally by noting any lymphopenia. Some divergence from normal values was found in 38 patients (64%) 14 children (24%) showed pathologically low immunoglobulin levels and 7 (12%) even a subtotal absence of one of the immunoglobulin fractions 8 of the cases (14%) also showed a reduction of gammaglobulin level In 20 children (34%) the level of one or more of the three major Ig classes was elevated All except one became clinically normal within a few years showing normal gamma or immunoglobulin levels when tested The tuberculin test was positive in all patients The length and course of the disease in these cases was similar to that in patients with normal test values Gammaglobulin therapy did not have any beneficial effect Consequently it is concluded that although humoral immunological defects may be one aetiological factor they do not play any major role in the course of chronic otitis media in children

In order to study the role of immunological defects in children with chronic otitis media we examined 59 children who had been treated for long standing middle-ear infection in our clinic during the period 1966–1973 The study included (Table I) the determination of serum gammaglobulin level by paper electrophoresis and of serum immunoglobulins IgG IgA and IgM by the single radial immunodiffusion method (Mancini et al 1965) When indicated the examination was repeated once or several times over a period ranging from one month to 5 years Since all the children had received Calmette (BCG) vaccination during their first months of life the validity of the cell bound immune system could be evaluated by the tuberculin test In addition, any signs of lymphopenia were noted

The mean age of the children when the immunological system was tested was 25 months (Table II), the range being from 4 months to 13 years In addition to conservative therapy, 46 of the patients were mastoidectomized at about the same time as the immune system was tested A further 11 children were adenoidectomized and in only 2 cases were no operative measures necessary

Some deviation from the normal values was found in 38 patients i e in 64% (Tables III–IV) 14 children (24%) showed pathologically low immunoglobulin levels and 7 of them even lacked one of the immunoglobulin fractions when examined in this way The missing fraction was IgA in 4 cases and IgM in 3 However, the single radial immunodiffusion method used fails to detect trace amounts of im-

Table I *Diagnostic tests*

[illegible]

Determination of gammaglobulin levels by paper electrophoresis (compared with normal values with ±1 96 standard deviations presented by Oberman et al 1956)
Determination of the amount of lymphocytes in peripheral blood (less than 1 500 lymphocytes/mm³ was noted as lymphopenia)
Evaluation of tuberculin reactivity (cell bound immunity)

Table II *Series of patients studied*
Mean age when first tested 25 months

	No of patients
Boys	42
Girls	17
Total	59
Mastoidectomized	46
Only adenoidectomized	11
No operation	2

munoglobulins, which probably remained in these cases, as evidenced by the subsequent return to normal of the clinical situation and immunoglobulin values Consequently, these cases could be included among those of reduced immunoglobulins This gives altogether pathologically low IgA in 10 cases, IgM in 6 and IgG in 4 cases Of the cases, 12 returned to normal clinically within 3 years, and in 6 of these immunoglobulins were re-examined later, showing normal values Of the 14 children, 8 had also reduced gammaglobulin levels Of these, 5 became normal within 3 years

One or more of the three major immunoglobulin classes were elevated in 20 patients (Table IV), i e 34% Altogether IgG was elevated in 7 cases, IgA in 14 and IgM in 13 17 of these returned to normal clinically within 3 years

Five children gave negative tuberculin test All these 5 however had been tuberculin positive earlier pointing to a functioning T-cell system

Four children had relatively mild (700–

Table IV *Elevated immunoglobulin levels*

	No of patients
IgG elevated	7
IgA elevated	14
IgM elevated	13
Total elevated Ig levels	20

1300/mm³) lymphopenia, but only 2 of these showed lymphopenia repeatedly in the tests, one of them had malignant histiocytosis and the other lipoid nephrosis as the basic disease

To summarize the clinical course as measured by the length of ear drain (Table V) The mean draining time in the total series was 29 months, and that in the children without defects 26 months Among those with reduced immunoglobulins the time was 30 months, while in the children with elevated immunoglobulin fractions the ear drain continued for 25 months on average

Gammaglobulin substitution (Table VI) was given to 7 children, though this did not lead to any shortening of the mean draining time

On surveying the present series we can see that the reductions in immunoglobulins are relatively frequently encountered in children with chronic otitis media The most common finding was low serum IgA value, the significance of which however, in small children is questionable (Immonen, 1967) Anyway, the immunoglobulins, and the clinical situation in general returned to normal within a few years The course of the disease was, in fact, not different from that in children with

Table III *Reduced immunoglobulin levels*

	No of patients
IgG reduced	4
IgA reduced	10
IgM reduced	6
Total reduced Ig levels	14
Reduced Ig+Gamma	8

Table V *Length of disease (average draining time)*

Immunological finding	No of ears	Draining time (months)
Normal	21	26
Ig reduced	12	30
Ig elevated	20	25

Table VI *Effect of gammaglobulin therapy*

Immunological defect	Treated with gammaglobulin: No of cases	Treated with gammaglobulin: Length of disease (mos)	Not treated with gammaglobulin: No of cases	Not treated with gammaglobulin: Length of disease (mos)
Ig reduced	7	33	5	36

normal immunovalues The same was true of the relatively large number of children with elevated values for one or more major immunoglobulin classes The frequencies of the defects are in accordance with those reported by Sala et al (1968) for recurring infections of the respiratory tract and by Zonis (1968) for Indian children with chronic otitis media

The reason for the relatively frequent occurrence of low immunoglobulins and their spontaneous return to normal is unclear May be this is a manifestation of delayed maturation of immunoglobulins in these patients which in such cases may predispose the children to repeated infections The elevated values on the other hand may probably result from increased stimulation or from mechanisms which serve to compensate for qualitatively inadequate immunoglobulins as proposed by Berg et al (1971) However the similarity in the course of the disease irrespective of whether there is shown to be any immunological defect and the ineffectiveness of gammaglobulin therapy points to the fact that although humoral immunological defects may constitute one aetiological factor they do not play any major role in the course of chronic otitis media in children

ZUSAMMENFASSUNG

59 Kinder mit chronischer Mittelohrentzundung wurden hinsichtlich moglicher immunologischer Defekte untersucht durch Feststellung von Gammaglobulin und Immunoglobuline IgG IgA und IgM des Serums sowie durch einen Tuberkulintest und weiter durch Aufweis jeder Lymphopaenie Gewisse Abweichungen von normalen Werten wurden bei 38 Patienten (64%) gefunden 14 Kinder (24%) zeigten pathologisch niedrige Immunoglobulin Spiegel und 7 (12%) sogar subtotale Abwesenheit eines der immunoglobulinen Bestandteile 8 der Fälle (14%) zeigten daruber hinaus eine Reduktion des Gammaglobulin Spiegels Bei 20 Kindern (34%) war der Spiegel von einer oder mehr der drei wichtigsten Ig Klassen erhoht Alle außer einem waren uber Jahre hin klinisch normal zeigten also normale Gamma bzw Immunoglobulin Spiegel als sie untersucht wurden Der Tuberkulintest war bei allen Patienten positiv Lange und Verlauf der Krankheit waren in diesen Fällen ahnlich zu jenen von Patienten mit normalen Testwerten Eine Gammaglobulin Therapie hatte keinerlei positiven Effekt Daraus folgt daß obwohl humorale immunologische Defekte ein atiologischer Faktor sein konnen sie trotzdem keinerlei wichtigere Rolle spielen im Verlauf der chronischen Mittelohrentzundung bei Kindern

REFERENCES

Berg D E Larsen A E & Yarington C T Jr 1971 Association between serum and secretory immunoglobulins and chronic otitis media in Indian children *Ann Otol Rhinol Laryngol 80* 766

Immonen P 1967 Levels of the serum immunoglobulins A G and M in the malabsorption syndrome in children *Ann Paediat Fenn 13* Fasc 4

Mancini G Carbonara A O & Heremans J R 1965 Immunochemical quantitation of antigens by single radial immunodiffusion *Immunochemistry 2* 235

Oberman J W Gregory K O Burke F C Ross S & Rice E C 1956 Electrophoretic analysis of serum proteins in infants and children I Normal values from birth to adolescence N *Engl J Med 255* 743

Sala O Gravina E & Sanvitale G 1968 Abnormalities of serum immunoglobulins and recurring infections of the respiratory tract *Pract Otorhinolaryngol 30* 353

Zonis R D 1968 Chronic otitis media in the South western American Indian II Immunologic factors *Arch Otolaryngol 88* 46

Acta Otolaryngol 82 196–198, 1976

THE IMMUNOLOGICAL FUNCTION OF THE ADENOID

The Proportions of T and B Cells

Britta Rynnel Dagoo

From the Department of Otolaryngology and the Transplantation Laboratory Karolinska Institutet and Huddinge Sjukhus Huddinge Sweden

Abstract The proportions of T and B cells in lymphocyte suspensions prepared from peripheral blood and adenoids from children undergoing adenoidectomy were determined using Ig fluorescence and E rosetting techniques The proportion of B cells is higher in the adenoid than in the blood and the proportion of T cells is correspondingly lower Preliminary results of stimulations of adenoid lymphocytes by natural antigens such as N catarrhalis indicate that both mitogenic and antigenic responses are induced Separation of lymphocyte suspensions on leucopac and subsequent stimulation by *N catarrhalis* and PHA result in abolition of the response to *N catarrhalis* indicating that reaction to this natural antigen is a B cell function

In the vast literature on immunology, there is a large number of studies dealing with the lymphocytes separated from peripheral blood However it is well known that there exist subpopulations of lymphocytes residing in lymphoid organs other than the blood It is therefore of great interest to study these other lymphoid organs The adenoid is a particularly convenient tissue since it is routinely removed from children, and as Morag Ogra (1975) mentioned in their recent review very little is known of the lymphoid populations of the adenoid

In our study adenoids from 23 children with the common indications for adenoidectomy, particularly symtoms of obstruction were excised The tissue was cut into small pieces and passed through a wire screen in order to obtain a single cell suspension This was then separeted on a Ficoll Isopaque gradient to obtain a suspension of lymphocytes

A large proportion of B cells maybe all of them, have surfaceimmunoglobulin which is the receptor for antigen These immunoglobulins are demonstrable by using an immunofluorescence technique with reagents such as fluorescein labelled antiimmunoglobulin, which is added to a suspension of lymphocytes Provided the reaction is carried out in the cold, the surface Ig will not move in the surface plane, thus inhibiting interiorization or endocytosis and will react only with surface compounds forming patches Under the microscope, the B lymphocytes are surrounded by these patches

T cells, on the other hand do not have conventional surface Ig and can be identified by their capacity to react with sheep red blood cells (SRBC) forming rosettes The mechanism behind this binding is unknown and has no immunological implication Washed SRBC in fetal calf serum + a suspension of lymphocytes 2×10^6 is incubated for 30 min at 37°C The mixture is centrifuged the pellet carefully resuspended, and lymphocytes with and without rosettes are counted

Fig 1 represents mean values of B and T cells in adenoid and peripheral blood There are 25% B cells in peripheral blood and this is in fact higher than in adults Similar results have been found by other groups Compared with the values for B cells in the blood those in the adenoid are much higher about 50% and the T cell values are lower

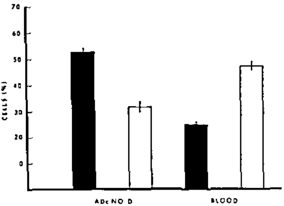


Fig 1 B cells (■) and T cells (□) in adenoid and peripheral blood

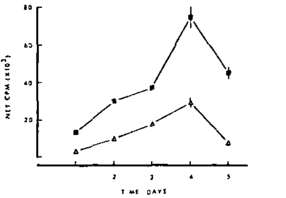


Fig 2 Stimulation of adenoid lymphocytes (2.5×10^6) with *N catarrhalis* (1/5 diln ■ and 1/50 diln △)

We then decided to study the response of adenoid lymphocytes to natural antigens One of the best known methods for measuring lymphocyte response to antigens and mitogens is the DNA stimulation technique Lymphocytes are placed in culture with the antigen, and the degree of proliferation is measured by incorporation of tritiated thymidine added to the culture 24 hours prior to harvesting The radioactivity is measured using a scintillation counter The data are expressed as arithmetical means ± standard errors of four individual cultures

The antigens used in this study are bacteria, which have been implicated in infections of the upper respiratory tract and in the middle ear of children, *H influenzae*, *D pneumoniae* and *N catarrhalis* One problem is to find out how to prepare the antigens and to define which antigenic components are responsible for the effects observed

Since in our preliminary experiments *N catarrhalis* a gramnegative bacterium gave us the most interesting results we decided to concentrate on this From *N catarrhalis* we have a heat killed suspension consisting of both cells and supernatant containing 2×10^9 cells/ml

Fig 2 is a kinetic curve of two different cell-concentrations of *N catarrhalis* 4×10^7 and 4×10^6 cells/ml As is quite typical with an antigen, there is a peak on day 4, but on day 2 there is a good response compared with the other antigens we have studied This may indicate the presence of a component having mitogenic properties either in the cells or in the supernatant Upon exposure to an antigen, stimulation of one clone of cells occurs In the case of a B cell clone one specific type of immunoglobulin is produced

Upon exposure to a mitogen, on the other hand a stimulation of many clones occurs and a nonspecific production of immunoglobulins is produced

The possible presence of a mitogenic component is most interesting since although the T cell mitogens, conA and phythaemagglutinin (PHA) elicit a high response from adenoid cells, the routinely used B cell mitogens LPS

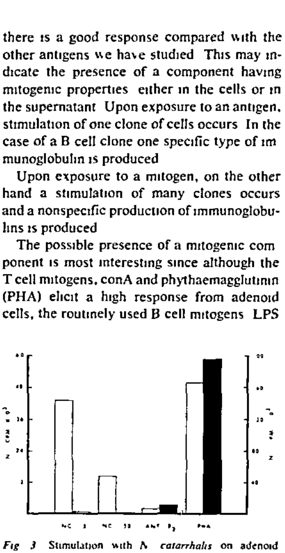


Fig 3 Stimulation with *N catarrhalis* on adenoid lymphocytes separated on leucopac (■) and unseparated (□)

and PPD elicit a poor response and antibeta$_2$-microglobulin a variable response We then decided to determine which population of cells were responding to *N catarrhalis* on day 2 An adenoid was prepared in the usual fashion and the cell suspension was then passed through a nylon wool column wich retains B cells Ig positive cells. B cells were then counted before and after separation The values were 50% before and 2% after separation Stimulation of *N catarrhalis* was then carried out on unseparated and separated cells As can be seen from Fig 3, there is a good stimulation before and no stimulation after separation, indicating that the cells which are reacting with *N catarrhalis* were retained in the column The PHA value is higher after separation, as would be expected since the suspension is then enriched for T cells

The tonsils and the adenoid act as a barrier against infection Friedmann's electron microscopic studies (1972) on adenoids have shown the presence of special reticulum cells with folded surfaces dendritic cells whose function is to retain antigen The high proportion of B cells in the adenoid, and the fact that these cells can be stimulated by antigens and mitogens raise the question as to the extent of immunoglobulin production in the adenoid With immunofluorescence studies Ahlqvist et al (1974) found intracellular IgM IgG and IgA in a few adenoids

A great clinical problem in children between the ages of 6 months to 3 years is recurrent otitis media A study by Branefors Helander et al (1975) has shown that hypogammaglobulinaemia is rarely found in this agegroup On the other hand, high levels are often detected However bacteria are often found in the naso pharynx in the absence of clinical infection

We intend to continue working with adenoid cells from children with well defined histories of infections extending our studies on stimulation of B lymphocytes with natural antigens and developing methods to the determination of the extent of local production of immunoglobulin in the adenoid

ZUSAMMENFASSUNG

Wir haben das Vorkommen von T und B Zellen in Lymphocytsuspension gewonnen aus Blut und Nasopharynxtonsillen von Kindern die Adenotomie durchgemacht haben bestimmt Dabei wurden Immunofluoreszenz und Rosettentechnik benutzt Das Vorkommen von B Zellen ist großer in den Nasopharynxtonsillen als im Blut und das Vorkommen von T Zellen ist entsprechend geringer Die vorlaufigen Resultate mit Stimulation von Nasopharynxlymphocyten mit *N catarrhalis* haben sowohl antigenische als auch mitogenische Stimulation gezeigt Nach Separation von Lymphocyten an Leucopac wurde keine Stimulation von *N catarrhalis* erhalten Die Reaktion ist demnach eine B Zellen Funktion

REFERENCES

Ahlqvist J Rasanen J A Antoniades K & Wallgren G R 1974 On morphology of lymph node immune response and follicular fibrin IgM IgG and IgA in lymphatic tissue *Ann Clin Res 6* 50

Branefors Helander P Dahlberg T & Nylen O 1975 A clinical bacteriological and serological study of children with frequent episodes of acute otitis media Thesis Goteborg

Friedmann I Michaels L Gerwat J & Bird E S 1972 The microscopic anatomy of the nasopharyngeal tonsil by light and electronmicroscopy *ORL 34* 195

Greaves M & Janossy G 1972 Elicitation of selective T and B lymphocytes responses by cellsurface bindig ligand *Transplant Rev 11* 87

Morag A & Ogra P 1975 Immunologic aspect of tonsils *Ann Otol Rhinol Laryngol* 19-84 37

Roitt I 1974 *Essential Immunology* Blackwell Scientific Publications

Acta Otolaryngol 82 199–201, 1976

PHENYTOIN INDUCED DEPRESSION OF SALIVARY IgA

J A Aarli

From the Department of Neurology School of Medicine University of Bergen Bergen Norway

Abstract Phenytoin treatment may induce a deficiency both of serum and salivary IgA In such cases large amounts of free SC are found in saliva

IgA is the predominant Ig in saliva The secretory IgA is of importance in the immune defence against potentially pathogenic organisms

Phenytoin, the most commonly employed anti-epileptic drug, depresses the serum concentration of IgA to subnormal levels in 20–25% of patients taking anticonvulsants (Aarli & Tønder, 1975) In some individuals even an immunodeficiency state (IgA<0 05 mg/ml) is induced (Aarli, 1976) Secretory IgA differs from IgA of serum in certain physicochemical characteristics Furthermore it is mainly locally synthetized and not passively transferred from the blood The aim of the study reported here was to investigate whether phenytoin treatment also depresses synthesis of secretory IgA

MATERIALS AND METHODS

Sera

Sera were taken from patients with epilepsy treated in the Department of Neurology Sera from healthy individuals within the same age groups as the patients served as controls (normal sera) A standard serum from Behringwerke AG Marburg Lahn West Germany served as reference serum for all immunoglobulin (Ig) determinations Rabbit antisera to IgA, IgM, IgG and to free secretory component (SC) were purchased from Behringwerke

Saliva

Whole saliva was collected at the same time as blood was drawn It was cleared by centrifugation at 1 700 *g* for 20 min and stored at −20°C until used

Ig determinations

Immunodiffusion plates were purchased from Behringwerke For sera, Tri Partigen and S Partigen plates were employed Ig concentrations in saliva were determined using LC Partigen plates Various dilutions of standard serum were always included for each plate In double diffusion studies, 1% agar was employed

RESULTS

IgA in saliva

IgA concentrations were determined in saliva from 10 healthy individuals from 10 patients taking anti-epileptic drugs and with normal serum IgA and from 10 patients with epilepsy and low serum IgA (below 0 15 mg/ml) Serum IgA was employed as standard In the normal samples the IgA concentrations varied between 6 and 9 mg/100 ml In saliva from epileptic patients with normal serum IgA, the IgA concentrations in saliva varied between 1 and 10 mg/100 ml In saliva from ep tients with low serum IgA the saliv

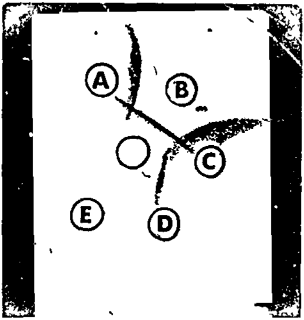


Fig 1 Double diffusion on agar plate Centre well saliva from a patient with epilepsy and IgA deficiency Wells A–E anti IgA anti SC anti IgG PBS and anti IgM Distinct lines are seen between centre well and anti SC and between centre well and anti IgG (Precipitation lines between individual antisera are due to the presence of Ig used for absorption of antisera)

concentration was considerably lower IgA was not detected in two samples, and was below 1 mg/100 ml in the remaining eight The two patients with undetectable salivary IgA had pronounced gingival hyperplasia

One IgA-deficient saliva was selected for further studies In double diffusion on agar plates, no precipitation lines occurred against anti IgA and anti IgM while distinct lines were seen against anti SC and anti IgG (Fig 1) Immuno electrophoresis showed mobility as compared with that of free SC

Effect of phenytoin upon salivary IgA

IgA concentrations were determined in serum and saliva in 5 patients with epilepsy before any drug treatment was given, and at intervals during the first year of treatment The Ig levels were normal, both in serum and saliva before drug treatment was given

In 2 patients, the IgA concentrations showed only moderate fluctuations within the normal range, both in serum and saliva In one patient, there was a fall in serum IgA with no concomitant change of salivary IgA In two patients, there was a distinct depression of IgA concentrations, both in serum and saliva (Table I)

DISCUSSION

One side effect of anti epileptic treatment with phenytoin is a depression of the serum concentration of IgA This occurs in 20–25% of patients on anticonvulsant therapy (Aarli & Tønder, 1975, Aarli, 1976)

IgA is the predominant Ig in saliva There is, however, not always a correlation between the IgA concentration in serum and in saliva Although the lack of correlation is predominantly seen in hypergammaglobulinaemia with elevated serum IgA certain patients may have depressed serum concentrations of IgA to

Table I *Effect of phenytoin upon IgA concentrations in serum and saliva in 3 patients (I II III)*

	Before treatment	During phenytoin treatment for		
		1 month	3 months	6 months
I				
Serum IgA (mg/ml)	2 27	1 57	0 98	0 98
Salivary IgA (mg/100 ml)	2 6	3 8	2 2	2 2
II				
Serum IgA (mg/ml)	1 57	0 82	0 63	0 77
Salivary IgA (mg/100 ml)	3 5	8 5	1 7	2 2
III				
Serum IgA (mg/ml)	1 51	0 72	0 02	0 02
Salivary IgA (mg/100 ml)	8 3	6 2	1 2	<1 0

gether with normal salivary IgA (Tomasi & Bienenstock, 1968)

The results of the present study have, however, shown that some patients receiving anti epileptic treatment have low concentrations of IgA both in serum and in saliva Thus, in 2 patients with IgA deficiency and with gin gival hyperplasia no IgA was demonstrated in saliva Furthermore, longitudinal studies of salivary IgA before and during phenytoin treatment showed a distinct fall in the IgA concentration during treatment Accordingly, phenytoin treatment may induce a deficiency state also for secretory IgA

SC was demonstrated in great amounts in saliva from the 2 patients with IgA deficiency Free SC is present also in normal saliva (Brandtzaeg et al , 1970) Therefore, the effect of phenytoin upon salivary IgA must occur before the conjugation with SC

According to Brandtzaeg (1971), secretory IgM is not attached to SC Coelho et al (1974), however, demonstrated IgM linked to SC in saliva from an individual with selective IgA deficiency In the present cases, SC occurred in free form Glandular synthesis of IgM may occur in IgA deficiency, but there was no in crease of IgM in the two samples with IgA deficiency

IgG passes into saliva by simple diffusion (Brandtzaeg et al 1970) An increase of IgG was seen in saliva from 2 patients with gingival hyperplasia This finding may probably be explained by increased transfer of IgG through the inflamed mucosa and is therefore regarded as a consequence of the gingival hyperplasia

The biological consequences of secretory IgA deficiency are not known It is possible that IgA mediates protection of the surface epithelium and the teeth (Brandtzaeg et al 1970) The drug induced salivary IgA de ficiency may be a contributory factor in the pathogenesis of gingival hyperplasia Pre sumably, other factors, such as a direct effect upon connective tissue, are also implicated (Aas, 1963) However, further studies are needed to elucidate the role of these factors in the pathogenesis of gingival hyperplasia

ZUSAMMENFASSUNG

Therapie mit Phenytoin kann in einigen Patienten mit Epilepsie einen IgA Mangel sowohl im Serum als im Speichel verursachen In solchen Fallen kommt SC in freier Form im Speichel vor

REFERENCES

Aarli J A 1976 Drug-induced IgA deficiency in epi leptic patients *Arch Neurol 33* 296

Aarli J A & Tønder O 1975 Effect of antiepileptic drugs on serum and salivary IgA *Scand J Immunol 4* 391

Aas, E 1963 *Hyperplasia Gingivae Diphenylhydantoi dea* Scandinavian Univ Books Universitetsforlaget Oslo

Brandtzaeg P 1971 Human secretory immunoglobulins II Salivary secretions from individuals with selec tively excessive or defective synthesis of serum im munoglobulins *Clin Exp Immunol 8* 69

Brandtzaeg P Fjellanger I & Gjeruldsen S T 1970 Human secretory immunoglobulins I Salivary secre tions from individuals with normal or low levels of serum immunoglobulins *Scand J Haemat* Suppl *12* 1

Coelho I M Pereira M T Virella G & Thompson R A 1974 Salivary immunoglobulins in a patient with IgA deficiency *Clin Exp Immunol 18* 685

Tomasi T B & Bienenstock J 1968 Secretory immunoglobulins *Adv Immunol 9* 1

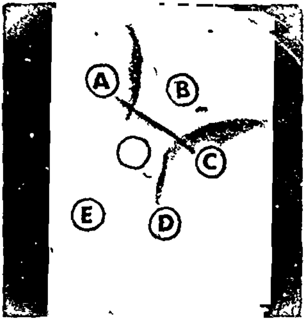


Fig 1 Double diffusion on agar plate Centre well saliva from a patient with epilepsy and IgA deficiency Wells A–E anti IgA anti SC anti IgG PBS and anti IgM Distinct lines are seen between centre well and anti SC and between centre well and anti IgG (Precipitation lines between individual antisera are due to the presence of Ig used for absorption of antisera)

concentration was considerably lower IgA was not detected in two samples and was below 1 mg/100 ml in the remaining eight The two patients with undetectable salivary IgA had pronounced gingival hyperplasia

One IgA-deficient saliva was selected for further studies In double diffusion on agar plates, no precipitation lines occurred against anti IgA and anti IgM while distinct lines were seen against anti SC and anti IgG (Fig 1) Immuno-electrophoresis showed mobility as compared with that of free SC

Effect of phenytoin upon salivary IgA

IgA concentrations were determined in serum and saliva in 5 patients with epilepsy before any drug treatment was given, and at intervals during the first year of treatment The Ig levels were normal, both in serum and saliva before drug treatment was given

In 2 patients, the IgA concentrations showed only moderate fluctuations within the normal range, both in serum and saliva In one patient, there was a fall in serum IgA with no concomitant change of salivary IgA In two patients, there was a distinct depression of IgA concentrations, both in serum and saliva (Table I)

DISCUSSION

One side effect of anti epileptic treatment with phenytoin is a depression of the serum concentration of IgA This occurs in 20–25% of patients on anticonvulsant therapy (Aarli & Tønder, 1975, Aarli, 1976)

IgA is the predominant Ig in saliva There is, however, not always a correlation between the IgA concentration in serum and in saliva Although the lack of correlation is predominantly seen in hypergammaglobulinaemia with elevated serum IgA, certain patients may have depressed serum concentrations of IgA to

Table I *Effect of phenytoin upon IgA concentrations in serum and saliva in 3 patients (I, II III)*

	Before treatment	During phenytoin treatment for		
		1 month	3 months	6 months
I				
Serum IgA (mg/ml)	2 27	1 57	0 98	0 98
Salivary IgA (mg/100 ml)	2 6	3 8	2 2	2 2
II				
Serum IgA (mg/ml)	1 57	0 82	0 63	0 77
Salivary IgA (mg/100 ml)	3 5	8 5	1 7	2 2
III				
Serum IgA (mg/ml)	1 51	0 72	0 02	0 02
Salivary IgA (mg/100 ml)	8 3	6 2	1 2	<1 0

Table II *Lymphocyte infiltrations in 37 patients with thyroid disease related to presence or absence of antibodies*

Lymphocyte infiltration	Antibodies	
	Present	Absent
Germinal centres	6	2
Crowds	3	0
A few scattered	2	9
None	0	15
Total	11/11	11/26

Histological examination of thyroid tissue removed at operation revealed the diagnoses shown in Table I The correlation between auto-immunity and histology, especially lymphoid infiltration, was as shown in Table II

Obviously, the determinations of antibodies in these 37 patients do not make diagnosis possible This is no surprise As early as 1958, two British investigators found thyroid antibodies in one third of a group of patients suffering from non toxic goitre or cancer of the thyroid In 1962, Hjort & Mogensen examined 132 healthy persons and found that 3% had thyroid antibodies A disputed question is how often antibodies to the thyroid gland are found in patients suffering from diseases not involving the thyroid gland or immune system In 1963, Hjort found antibodies in 11% of a group of 180 patients—a percentage which is considerably higher than those reported by other investigators This disparity is probably due to different methods of antibody determination and incomparable patient groups the latter often varying widely as regard age and sex As previously mentioned determination of antibodies against the thyroid is of no great diagnostic value in the individual patient titres may be equally high in patients with nodular goitre and Hashimoto's disease In the small group of patients included in this study, there was however, found evidence in support of the assumption that circulating antibodies against the thyroid gland correspond with lymphocyte infiltration in the gland, which is of considerable prognostic interest In 1949 Whitesell & Black showed a relation between lymphoid infiltration and the development of postoperative myxoedema This has been confirmed by several investigators

Apart from making it possible to exclude the diagnosis of Hashimoto's disease, the value of determination of the thyroid antibodies lies in their correspondence with lymphocyte infiltration, allowing an estimation of prognosis and potential risk at operation Patients found to have a high antibody titre should be checked for signs of chronic thyroiditis or Hashimoto's disease

ZUSAMMENFASSUNG

In den Jahren 1971–74 lagen insgesamt 88 Patienten mit Thyreoideaerkrankungen auf der Otologischen Abteilung des Finseninstitutes Bei 37 dieser Patienten die auf Thyreoideaantistoffe untersucht wurden konnte die Diagnose durch eine histologische Untersuchung des Thyreoideagewebes bestätigt werden Aus dem vorliegenden Material geht hervor daß das Vorhandensein von Thyreoideaantistoffen ein Ausdruck für den Grad der lymphozytaren Infiltration in der Thyreoideadruse ist und als solches für die Beurteilung der Prognose des Patienten und des potentiellen Operationsrisikos von Bedeutung ist

REFERENCES

Hjort T 1963 *Autoimmunisering ved thyreoidealidelser* Århus

Halberg P 1967 *Kronisk thyreoiditis* Copenhagen

Faber V 1975 Autoimmune sygdomme *Med komp* 11 udg

Roitt I M & Doniach D 1960 Thyroid autoimmunity *Brit Med Bull 16*

Whitesell & Black 1949 A statistical study of the clinical significance of lymphocytic and fibrocytic replacements in the hyperplastic thyroid gland *J Clin End 9*

Acta Otolaryngol 82 204–207, 1976

HUMAN AUTO-IMMUNE THYROIDITIS

A Biorklund and N Soderstrom

From the ENT Department and the Department of Medicine University Hospital Lund Sweden

Abstract Focal or diffuse infiltration of lymphoid cells in auto-immune thyroiditis destroys the parenchyma In the diffuse form (Hashimoto s disease) the clinical picture is characterized by fatigue and thyroid enlargement The diagnosis is settled by cytology All patients must be treated with thyroid hormones to avoid myxoedema Loss of immunological tolerance and cytotoxic immune reactions is discussed

The aim of this paper is to discuss some aspects of the most common form of thyroiditis, i e chronic thyroiditis with infiltration of lymphoid cells, also called lymphoid thyroiditis or auto-immune thyroiditis

In light microscopy of lymphoid thyroiditis the histopathology is characterized by diffuse or focal infiltration of lymphoid cells and destruction of the original parenchyma, usually resulting in the formation of more or less complete lymphoid tissue, with germinal centres and other evidence of a specific tissue differentiation

In clinical practice, the diffuse chronic thyroiditis (struma lymphomatosa or Hashimoto's disease) is fairly easily diagnosed and thus more or less loosely identified with the concept of auto immune thyroiditis in man, and to a certain degree equivalent to experimental or spontaneous thyroiditis in animals Modification of different factors in the experimental disease has proved to be of importance in the understanding of the clinical picture

The clinical picture of Hashimoto s disease is at first glance rather elusive It may be met at any age, also in children with a very strong female preponderance It is most frequently seen in middle-aged women with a slight sometimes moderate enlargement of the thyroid, which rarely attracts much attention The complaints are usually very diffuse—general fatigue may be present, but otherwise there is nothing characteristic in the history Local tenderness or pain in the thyroid do not belong to the picture of Hashimoto's disease

A moderately elevated sedimentation rate may direct attention to the thyroid The gamma globulins are slightly increased, and it is not uncommon to find a low monoclonal "spike" in electrophoresis Employing different methods, circulating organ specific auto antibodies can be detected against thyroglobulin, microsomal antigen, surface antigen, and the so called second colloid antigen (CA_2) Usually, in Hashimoto's disease and primary myxoedema, circulating auto antibodies against at least one of these thyroid antigens are increased With a lower frequency, similar antibodies are found in healthy relatives of patients with Hashimoto's disease Scintigraphy demonstrates areas of low uptake During the disease these areas migrate In the laboratory tests or the history of the patient vague or fluctuating indications of hypothyreosis may exist It is our experience that overt evidence of hypothyreosis is a late feature in Hashimoto's disease and is found only in a minority of the cases It is important to realize that the initial phase of the disease is clinically unknown

The diagnosis is finally settled by the detection of an enlarged thyroid and by the fine needle aspiration biopsy The cytological pic

ture is very characteristic the smear looks like a lymph node smear with full representation of lymph node cytology This includes lymphocytes, lymphoblasts, centroblasts, tingle body cells, sinus epithelium and abundance of plasma cells The follicular epithelium cells are scarce and may be difficult to find In half of the cases, the epithelium includes transformed follicular cells called Askanazy or Hürthle cells ("oxyphilic epithelium" of histology) These cells have a broad foamy looking cytoplasm due to a high content of mitochondria The Askanazy cells have a fine "dusty" granulation of deeply basophilic granules with asymmetric distribution in the cell body The cells also exhibit a conspicuous nuclear polymorphism, which may be mistaken for malignancy Papillary carcinoma of the thyroid can have an advanced degree of lymphoid metaplasia, difficult to separate from Hashimoto's disease Previously, we (Soderström & Biorklund 1973) stressed the diagnostic importance of some types of intranuclear inclusions in the diagnosis of papillary carcinoma These inclusions are never seen in the Askanazy cells

We wish to stress that the diagnosis of diffuse lymphoid thyroiditis or Hashimoto's disease should never be made on the basis of a single thyroid puncture, at least two punctures must be done at the examination and the cytological picture should be the same in both specimens The single aspirate may contain material from a single lymphoid focus and thus suggests a false diagnosis of diffuse thyroiditis

All patients with confirmed Hashimoto's disease should be treated with levothyroxin in full myxoedema dosis i e on the average, 0 2 mg daily The rational for this treatment is to prevent the insidious onset of clinical myxoedema a common outcome in the course of Hashimoto's disease Too often this myxoedema is detected after years of high cholesterol levels in the serum resulting in coronary sclerosis and sudden death when thyroid hormone treatment finally is attempted The detection of lymphoid thyroiditis at an early stage is of paramount importance in the prevention of coronary disease After induction of therapy the diffuse complaints usually disappear and the thyroid enlargement is reduced The patients should be checked once a year, and the therapy is life long

The basic lesion in thyroid auto immunity is loss of tolerance against thyroid antigens In healthy subjects Bankhurst et al (1973) could detect binding of ^{125}I labelled thyroglobulin only to B cells in the peripheral blood In Hashimoto's disease, Roberts et al (1973) found 5 to 6 times more B cells binding this radio-labelled antigen These findings lend support to the hypothesis that antigen reactive T cells are eliminated by a low dose of antigen to avoid an auto-immune reaction In auto-immunity T cells sensitization occurs and, involving B cells abnormal immune reactions are induced Subjects susceptible to auto-immune diseases may have immune-response genes (Ir genes) rendering their T cells abnormal, responsive to organ specific antigens released by unspecific minor organ trauma These Ir genes, supposed to be linked to histocompatibility loci would explain the inheritance of auto-immune diseases and the susceptibility of certain animal strains to the induction of experimental auto-immune thyroiditis (Braley Mullen et al , 1975)

In Hashimoto's disease three immune reactions, cytotoxic for the follicular cells have been discussed In vitro studies of Pulvertaft et al (1959) demonstrated a cytotoxic complement-dependent antibody in Hashimoto sera In similar test systems sensitized lymphoid cells but not sera from rats with experimental auto-immune thyroiditis (Biörklund 1964) and Hashimoto patients (Podleski 1972) were found to be cytotoxic to thyroid cells Ringertz et al (1971) and Calder et al (1973) described a third cytotoxic immune reaction the antibody mediated lymphocytotoxicity in experimental and human auto-immune thyroiditis The cytotoxicity inducing antibody involved in this reaction was of the IgG type

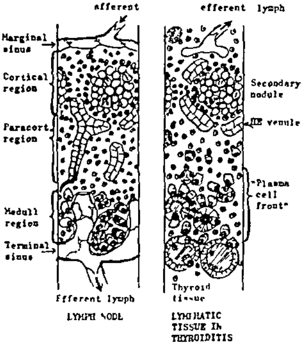


Fig 1 A comparison of the structural organization of a lymph node and lymphatic tissue in Hashimoto s disease cortex/germinal centre paracortical region/high endo thelium venules medulla/plasmocytic front

(γ_2) and could not be correlated to haemagglutinating antibody titres in the sera (Wasserman et al 1974) The lymphocyte in the anti body mediated lymphocyte cytotoxicity is uncommitted and does not belong to T or B cells subpopulations Recently, Calder et al (1975) discussed the characteristics of these cells K (killer) cells The cells lack immunological specificity, but have receptors for activated Fc fragments of antibodies The K cells acquire a high cytotoxic potential and immunological specificity by combining with auto antibody bound to thyroid cells or with circulating im mune complexes in antibody excess

The in vivo findings of spontaneous plasma cell dominating thyroiditis in chickens (Wick et al , 1970) and Buffalo rats (Noble et al , 1975) stress the role of B cells in auto immune thyroiditis The complete and sustained reversal of experimental auto immune thyroi ditis after cyclophosphamid therapy further stresses the role of B cells (Paterson & Drobish, 1975) This alkylating agent is supposed to have a cytolytic effect on proliferating B cells In Hashimoto s disease, Kalderon et al (1973) found plasma cell-associated electron dense deposits, similar to immune complexes, at the follicular basement membrane The same deposits, consisting of thyroglobulin C 3 and IgG, were also found in a case of multi centric papillary carcinoma of the thyroid (Kalderon et al , 1975)

Using thin-section histology in Hashimoto specimens we have found a massive invasion of plasma cells into follicular areas (Soderstrom & Biorklund, 1974) The invading lymphoid tissue in the thyroiditic specimens was organized as in a lymph node The medul la of a lymph node was represented in Hashi moto tissue by a plasmocytic front invading the follicles Plasma cells, but rarely lympho cytes, were often associated with splits in the follicular cell basement region The follicular cells close to the plasma cells were either de generated cells or Askanazy cells The para cortical region in thyroiditis corresponded to postcapillary high endothelium venules, which are easily mistaken for follicles Finally, the germinal centres of thyroiditis corresponded to the node cortex (Fig 1)

Our findings demonstrate that a massive in volvement of plasma cells occurs in Hashi moto's disease This may reflect the loss of immunological tolerance to thyroid antigens giving rise to expanding B cells clones, as discussed by Roberts et al (1973) The role of these plasma cells in the cytotoxic reaction is unclear, but recent finding in antibody medi ated lymphocyte cytotoxicity, as discussed above, may indicate their role

Human auto immune thyroiditis is a disease sometimes seen by the head and neck oncologist It may be difficult to differentiate from papillary thyroid carcinoma due to the intense immune reaction occurring in both diseases It is an instructive immunological disorder giving the clinician basic information of im munological processes, such as loss of tol erance and immune destruction in an organ specific auto immune manifestation

ZUSAMMENFASSUNG

Fokale oder diffuse Infiltration von lymphoiden Zellen in autoimmunen Thyreoiditen zerstort die Parenchyme In der diffusen Form (Hashimoto s disease) wird das klinische Bild von Mudigkeit und Vergroßerung von Glandula Thyreoidea charakterisiert Die Diagnose wird durch Cytologie gestellt Alle Patienten mussen mit Thyreoidea Hormonen behandelt werden um Myxodema zu verhindern Verlust von immunologischer Toleranz und cytotoxische Immunitätsreaktionen werden diskutiert

REFERENCES

Bankhurst A D Torrigiani G & Allison A C 1973 Lymphocytes binding human thyroglobulin in healthy people and its relevance to tolerance for autoantigens *Lancet I* 226

Biorklund A 1964 Testing in vitro of lymphoid cells from rats with experimental autoimmune thyroiditis *Lab Invest 13* 120

Braley Mullen H Sharp G C & Kyriakos M 1975 Differential susceptibility of strain 2 and strain 13 guinea pigs to induction of experimental autoimmune thyroiditis *J Immunol 114* 371

Calder E A Penhale W J & Irvine W J 1975 Killer cells and immune complexes in thyroid disease *Proc Roy Soc Med 68* 250

Calder E A Penhale W J McLeman D Barnes E W & Irvine W J 1973 Lymphocytedependent antibody mediated cytotoxicity in Hashimoto thyroiditis *Clin Exp Immunol 14* 153

Kalderon A E Bogaars H A & Diamond I 1973 Ultrastructural alterations of the follicular basement membrane in Hashimoto s thyroiditis *Am J Med 55* 485

— 1975 Immune complex deposition in thyroid carcinoma associated with chronic thyroiditis *Clin Immunol Immunopath 4* 101

Noble B Rose N R & Bigazzi P E 1975 Thyroid antibodies in spontaneous autoimmune thyroiditis of the Buffalo rat *Fed Proc 34* 835

Paterson P Y & Drobish D G 1975 Reversal of experimental allergic thyroiditis in cyclophosphamide treated rats *Clin Exp Immunol 20* 125

Podleski W K 1972 Cytotoxic lymphocytes in Hashimoto s thyroiditis *Clin Exp Immunol 11* 543

Pulvertaft R J V Doniach D Roitt I M & Hudson R V 1959 Cytotoxic effects of Hashimoto s serum on human thyroid cells in tissue culture *Lancet II* 214

Ringertz B Wasserman J Packalen T & Perlmann P 1971 Cellular and humoral immune responses in experimental autoimmune thyroiditis *Int Arch Allerg 40* 917

Roberts I M Whittingham S & Mackay I R 1973 Tolerance to an autoantigenthyroglobulin *Lancet II* 936

Söderström N & Biorklund A 1973 Intranuclear cytoplasmic inclusions in some types of thyroid cancer *Acta Cytologica 17* 191

— 1974 Organization of the invading lymphoid tissue in human thyroiditis *Scand J Immunol 3* 295

Wasserman J von Stedingk L V Perlmann P & Jonsson J 1974 Antibody-induced in vitro lymphocyte cytotoxicity in Hashimoto thyroiditis *Int Arch Allergy 47* 473

Wick G Kite J Cole R & Witebsky E 1970 Spontaneous thyroiditis in the obese strain of chickens III The effect of bursectomy on the development of the disease *J Immunol 104* 45

Acta Otolaryngol 82 208–211, 1976

WEGENER'S GRANULOMATOSIS, IMMUNOSUPPRESSIVE THERAPY

H. Thorkelsen and P Berdal

From the ENT Department, Rikshospitalet Oslo Norway

Abstract We present a study of 11 patients with Wegener s granulomatosis Seven of these were treated with Azathioprine (Imurel) combined with prednisone Of these, 4 patients rapidly improved after the treatment was started Atelectatic changes in the lungs disappeared A rapidly progressive renal failure was reversed, and renal function returned to normal Large lesions of the skin also improved The treatment has now lasted 2½–4½ years without interruption We have not had any serious side effects due to Imurel We believe that the good effect of the treatment is due to immunosuppression by the drugs

Wegener's granulomatosis is a rare disease of unknown aetiology Histologically, it is characterized by generalized focal necrotizing vasculitis with perivascular infiltration of lymphocytes At the present time, it is regarded as an auto immune disease with an unknown antigen Untreated, the patients die within 6 months (Shillitoe et al , 1974; Asin et al , 1972) Usually, the upper respiratory tract is first involved, later the disease is also found in the lower respiratory tract including the lung parenchyma and in the kidneys Other organs may also be involved, e g the orbita, middle ear, skin and joints Confronted with a disease affecting the upper respiratory tract, lungs and kidneys, the diagnosis of Wegener's granulomatosis should be considered (Gonzales & Ordstrand, 1973) A chronic otitis which does not improve during treatment may be a manifestation of Wegener's granulomatosis (Linthicum & Schwartzman, 1972)

TREATMENT

The principles of treatment in Wegener's granulomatosis have varied according to the current view of the nature of the disease

1. Before 1955, the treatment was purely symptomatic, and the patients usually died within 6 months after the onset of the disease

2 From about 1955 until the latter part of 1960, the treatment consisted of X-rays and steroids, and this led to great optimism No doubt, this treatment has improved the prognosis, but only to a slight extent The results of the treatment were mostly due to a general antiinflammatory effect

3 From the end of 1960, cytostatics have been used (Moeschlin, 1969, Skevas & Schmidt-Baumler, 1974, Teisberg & Enger, 1970, Høst, 1973) Very good results have been reported, and many patients have been able to start working again

Imurel (azathioprine) is a cytostatic drug (antimetabolite) with an immunosuppressive effect and few side effects Therapeutically, regression of the infiltrates and an improvement of the patient's general health may be achieved

Examples of the favourable results of Imurel treatment appear from the following case reports

Case no 5 (Table II)

A 38-year-old male factory worker, admitted to Rikshospitalet in May 1972 His complaints were pain in the nose, nasal stuffiness and frequent nose bleedings since November 1971 He had been unsuccessfully treated with antibiotics for rhinosinusitis by his family doctor On admission, an ulcerating infiltration in the upper dorsal part on both sides of the nose was found A biopsy specimen from the nasal mucosa showed ulceration and marked subacute inflammation with eosinophilia and giant

ɔle I *Wegener's granulomatosis Treatment with X-ray and/or corticosteroids*

e	Profession Sex	Age	Localisation of disease	Sympt duration (months)	Treatment and duration of treatment	Effect	Survival tin following treatment
	Clerk M	55	Oral and nasal mucosa lungs	46	Prednisone 20 mg/day 3 months prior to death	Progression of disease Dead of bronchopneumonia	3 months Dead
	Plumber M	63	Ramus mandibulae epipharynx lungs	28	X ray Jan 1963, 1 150 R mandibular field Feb 1963, 1 000 R mandibular field Oct 1963, 450 R mandibular field	Progression of disease Dead of lung abscess (May 1964)	16 months Dead
	Salesman M	52	Right tonsil	35	X ray Dec 1972 5 800 R Prednisone 60 mg/day reduced to 2.5 mg/day from Dec 1972	Great improvement Full time employed	30 months Alive
1	Teacher M	52	Mucosa of nose and maxillary sinus	9	X ray Dec 1960 1 800 R nasal field May 1961 1 800 R nasal field Prednisone 40 mg/day Dec 1960–May 1961	No improvement No improvement Dead 1 7 61	8 months Dead

cells, interpreted as Wegener's granulomatosis X-ray showed atelectasis of the posterior basal segment in the left lung Bronchoscopy Papillomatous tumour occluding the lumen immediately peripheral to the opening of the apical bronchus of the left lower lung Later he got haematuria, proteinuria, isosthenuria Serum creatinine increased to 6 1 mg/dl Creatinine-clearance fell to 29 ml/min Biopsy of the kidneys showed proliferative and interstitial inflammatory changes and fibrinoid necrosis Haemoptysis occurred Rapidly deteriorating kidney failure followed Serum electrophoresis showed increased $alpha_1$ and $alpha_2$ globulin and slightly increased immunoglobulin G and A In June 1972, treatment started with Imurel 50 mg × 5, and prednisone 5 mg × 2, daily Gradual improvement was obtained, and 5 months later the serum creatinine was 1 6 mg/dl The creatinine clearance increased to 140 ml/min A repeated biopsy from the kidney showed sclerosis of the glomeruli and great changes as compared to the first biopsy Repeated X-ray showed almost normal lungs Having been disabled for 1½ years, he now returned to work and has later been fully employed The lung function is normal The administration of 10 mg prednisone and 225 mg Imurel daily has been continued

Case no 7 (Table II)

A 41-year-old female nurse was admitted to Rikshospitalet in October 1972 Her complaints were Since August 1972 pain in the left cheek radiating to the nose and nasal stuffiness on the left side On admission a swelling on the left cheek was found and the left eye was dislocated laterally X-rays showed an infiltration in the left lung and exudate in the pleura Later she had a blood-tinged nasal secretion She presented a tumour like infiltration in the nose A biopsy specimen showed changes consistent with Wegener's granulomatosis She also had haematuria The serum creatinine was 2 1 mg/dl She was ill and confined to bed In November 1972, Imurel in a daily dose of 50 mg × 3 and prednisone, 10 mg × 3, were administered The general status rapidly improved and the X-ray changes in the lungs gradually decreased SR decreased from 115 mm to 23 mm in the course of a few months After 6 months in the spring of 1973, she was again f

Table II *Wegener's granulomatosis. Treatment with cytostatics*

Case no	Profession Sex	Age	Localisation of disease	Sympt duration (months)	Treatment	Duration of treatment
1 *LH*	*Glazier* *M*	*59*	Oral mucosa skin of penis and face, lungs	96	A X-ray B Imurel 150 mg/day Prednisone 15 mg/day	A *1966, 67, 70, 71* B Last 3 months before death
2 LI	Upholsterer M	63	Orbital tissue, kidneys lungs	34	A Prednisone 40 mg/day Imurel 150 mg/day B Prednisone 80 mg/day Natulan 200 mg/day Cyclophosphamid 1 000 mg i v weekly Vinblastin 10 mg i v weekly C Cyclophosphamid 50 mg/ *day oral* Prednisone 15 mg/day D No cytostatics	A 6 months B 3 sequences each of 2 weeks, interval 1 month C 2 months D Last year of life
3 *TN*	*Typist* *F*	*28*	Nasal and palatal mucosa Skin of leg tonsils	36	Imurel 200 mg/day *Prednisone 60–10 mg/day*	34 months continuous
4 AO	Shop girl F	65	Maxillary sinus, orbital tissues	54	Imurel 200 mg/day Prednisone 10 mg/day	48 months
5 LL	Factory worker M	38	Nasal mucosa lungs, joints, kidneys	42	Imurel 250 mg/day Prednisone 10 mg/day	36 months continuous
6 IJH	Housewife F	55	Lungs, kidneys	4	Imurel 75 mg/day Prednisone 80 mg/day	4 days prior to death of renal failure
7 EHT	*Nurse* F	*41*	Nasal mucosa and bone, lungs, kidneys	*36*	*Imurel 150–300 mg/day* Prednisone 10–50 mg/day	*32 months continuous*

ployed The local immune response, as studied by immunhistochemical methods, was not different *from that of an unspecific chronic* inflammation The B and T lymphocytes studied by marker technique and in vitro function test *were normal The phagocytosis of the* granulocytes was normal The former patient is still fully employed (January 1976)

During the past 15 years 11 patients with Wegener's granulomatosis have been admitted to Rikshospitalet, the Ullevaal Hospital, and to the *Nordland Sentralsykehus, Bodø* Among these, 7 patients have been treated with Imurel and prednisone The 4 other patients *(Table I) were given X-rays and/or* prednisone treatment Before the diagnosis, all patients had antibiotic treatment because an infectious inflammation was suspected

The X-ray treatment had delayed the progression of the disease It has also reduced the pain, and the general health has improved All *in all, the palliative effect* has been satisfactory Imurel combined with prednisone has been administrated to 7 patients (Table II) In *4 of the cases, this treatment* had a very good effect, although the patients probably have not been cured Two of these patients have al*ready been discussed (patients 5 and 7)* Imurel, 200–300 mg daily, has been given, and these patients have now been treated for 2½–4½ *years The treatment is continued* Side effects necessitating discontinuation of the treatment has occurred only once in a case in *which Imurel treatment was stopped* for 2 weeks due to leucopenia (2 000/ml³) The treatment has later been continued for 8 months *Side effects of prednisone, like osteoporosis* or moon face, have occasionally occurred In

ffect	Survival time following treatment
ood first years rogression of disease	81 months Dead
o improvement	26 months Dead
o improvement	
o improvement	
ogression of disease	
nprovement	33 months Alive
reat improve ment Full time mployed	48 months Alive
reat improve ment Full time employed	36 months Alive
lo improvement	8 days Dead
reat improve ment Full time employed	31 months Alive

3 cases, there was no definite improvement after treatment with Imurel and prednisone One reason for the lack of effect probably was that the disease had reached an advanced stage when the treatment was commenced

Wegener s granulomatosis like other diseases, may have different rates of progression and consequently show varying clinical manifestations in different patients This may be one of the reasons why the results of the treatment vary Another possibility is that there may be different types of the disease with special localisations and different rates of progression (lethal granuloma of the midline Steward s granuloma malignant granuloma)

The treatment of Wegener s granulomatosis with Imurel and prednisone is based partly on theoretical and hypothetical pathogenetic considerations and partly on experience In some patients with advanced disease, but not hopeless so, a good palliative effect has been obtained The manifestations in the kidneys lungs and respiratory tract have improved The side effects (leucopenia osteoporosis) have been few and has not necessitated delay of the treatment except for a very short period The treatment should be continued permanently

ZUSAMMENFASSUNG

Es werden elf Patienten mit Wegeners Granulomatose erwahnt von denen sieben mit Azathioprine (Imurel) kombiniert mit Prednison behandelt wurden Vier dieser Patienten zeigten eine schnelle Verbesserung gleich nach Beginn der Behandlung Atelektatische Lungenverande rungen verschwanden Ein fortschreitendes Nierenversagen horte auf und die Nierenfunktion normalisierte sich Großere Hautschaden heilten aus Die Behandlung hat inzwischen 2½–4½ Jahre gedauert und wurde nicht abgebrochen Wir haben keine ernste Nebenwirkungen von Imurel gesehen Man nimmt an daß der gute Behandlungserfolg auf einer medikamentell hervorgerufenen immunosuppressiven Wirkung beruht

REFERENCES

Asin H R G Wagenaar J P M & Swierenga J 1972 The mitigated form of Wegener s disease *Scand J Respir Dis 53* 367

Domarus H v 1973 Wegenersche Granulomatose als Ursache für Kiefer-Gesichts Schmertzen *Z Laryngol Rhinol Otol 52* 901

Gonzales L & Ordstrand H S 1973 Wegener s granulomatosis *Rad ology 107* 295

Harrison D F N 1974 Non healing granulomata of the upper respiratory tract *Br Med J IV* 205

Høst H 1973 Cytostatika og cytostatisk terapi *Tidsskr Nor Laegeforen 93* 1857

Jørgensen K E 1970 Immunosuppressiv behandling af Wegener s granulomatose *Ugeskr Laeger 132* 1549

Linthicum F H & Schwartzman J A 1972 Wegener s granulomatosis appearing initially as otitis media *Trans Am Acad Ophthalmol Otolaryngol 72* 301

Moeschlin S 1969 Grenzen und Möglichkeiten der immunosuppressiven Therapie *Therapiwoche 19* 1

Rørdam P & Hansen L A 1971 Wegener s granulomatosis *Dan Med Bull 18* 43

Shillitoe E J Lehner T Lessof M H & Harrison D F N 1974 Immunological features of Wegener s granulomatosis *Lancet I* 281

Skevas A & Schmidt Baumler U 1974 Die Behandlung der Wegenerschen Granulomatose mit Immunosuppressiva und Kortikosteroiden *Laryng l Rhinol Otol* (Stuttg) *53* 674

Teisberg P & Enger F 1970 Immunosuppressive therapy in Wegener s granulomatosis *Acta Med Scand 187* 7

Wegener s granulomatosis 1972 *Lancet II* 51)

Acta Otolaryngol 82 212–215, 1976

IMMUNOLOGICAL FINDINGS IN A CASE OF COGAN'S SYNDROME

S Edstrom and A Vahlne

From the Department of Otolaryngology Central Hospital Molndal and the Department of Virology Institute of Medical Microbiology University of Goteborg Goteborg Sweden

Abstract The immune system of a case of Cogan's syndrome was investigated A transient depression of the cell mediated immunity was found Thus during the acute stage of the disease, there was a depression of the cutaneous delayed hypersensitivity reaction to PPD and a decrease in the number of T cells There were also signs of complement consumption A possible pathogenesis based on immune complexes due to a preceding viral infection is discussed

In 1945, David Cogan published four cases of nonsyphilitic interstitial keratitis and vestibulo auditatory symptoms (Cogan's syndrome) Since then, about 60 cases have been reported The aetiology of the syndrome is unknown However, in earlier reports (Albrite & Resnick, 1961, Fisher & Hellstrom, 1961), vascular alterations have been found corresponding to those in systemic disease The purpose of the present investigation was to examine the immune system of a patient with Cogan's syndrome and evaluate the possibility of immune complex aetiology

CASE HISTORY

A 15-year-old girl had in association with a common cold experienced the onset of tinnitus, dizziness and ocular pain Two weeks later she noticed a hearing loss in her left ear and was admitted to hospital The patient had previously been healthy At the age of 7 she was BCG vaccinated and when tested six years later the PPD reaction (0 1 ml, 2 TU/ml) was positive

Ophthalmological examination

The eye lids were moderately oedematous and the bulbs were red The right corneal parenchyma showed a peripheral opaquefication and the corneal epithelium was diffusely oedematous on both sides Media and eye grounds were normal One week after admission to hospital the left corneal parenchyma was also opaquefied

Otoneurological examination

The hearing threshold level fell on both sides (Fig 1) The stapedius reflex test showed no elicitation at 120 dB Glycerol test was negative There was no nystagmus and the caloric test of vestibular function showed no reaction on either side

Routine laboratory examinations

The sedimentation rate and white cell count in blood were elevated The polymorphonuclear percentage ranged from 58 to 77 per cent Platelets were 550000 per mm³ Fibrinogen was elevated (6 63 g/l) Other routine laboratory investigations (including cytology of bone marrow, cytology and electrophoretic pattern of spinal fluid, function tests of heart, liver, thyroid and kidney, muscle biopsy, X-ray ex-

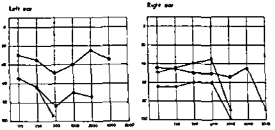


Fig. 1. Hearing threshold levels during the first 6 days after admission to hospital. ● admission. ✱ 3 days after admission. • 6 days after admission.

amination of heart, lungs, ears, porus acusticus and teeth) were normal.

METHODS AND RESULTS

Lymphocyte function tests

In vivo tests of the cutaneous delayed hypersensitivity reaction to 0.1 ml PPD (2 TU/ml) were performed. Lymphocyte stimulation tests were performed with the T cell mitogens Phytohaemagglutinin P (PHA) (Difco Laboratories, Detroit, Michigan) and Concanavalin A (ConA) (Miles Yeda Ltd, Rehovoth, Israel) as described by Mobacken & Lindholm (1974). Quantitation of T and B lymphocytes in venous blood was performed with the rosette-formation test described by Jondal et al. (1972). Inhibition tests of leucocyte migration in the presence of Monilia albicans and PPD extracts were performed using the polythene capillary tubing method of Hughes (1972).

When tested five weeks after the onset of the disease, there was no cutaneous delayed hypersensitivity reaction to PPD. The patient was tested one year later, and at that time an indurated and reddened zone of 20 mm in diameter at the site of the PPD injection was noted after 48 hours (Table I). The percentage of T cells was subnormal during the first 6 weeks of the disease (Table I). No impairment of the qualitative in vitro T cell function was noted. Thus, the lymphocytes were normally stimulated with the mitogens tested and leucocyte migration was inhibited in the presence of PPD and *M. albicans* (Table I).

Other immunological investigations

The complement factors C3 and C4 were determined with single radial immunodiffusion (Mancini et al., 1965), using Partigen immunodiffusion plates (Behringwerke AG, Marburg/Lahn, Germany). In the two first sera collected 3 and 6 weeks after onset of the disease, subnormal values were obtained for C3

Table I. *Lymphocyte function tests*

The cutaneous delayed hypersensitivity reaction to PPD (PPD), the percentage of T lymphocytes (T%) and B lymphocytes (B%), lymphocyte stimulation (LYS) with Phytohaemagglutinin (PHA) and Concanavalin A (ConA), leukocyte migration inhibition (LIF) in the presence of PPD and *Monilia albicans* (*M. alb.*)

Weeks after onset of the disease	5	6	11	56
In vivo test				
PPD[a]	Negative	NT[b]	NT	Positive
In vitro tests				
T% (N[c] 50–80%)	31%	32%	73%	NT
B% (N 10–20%)	12%	6%	23%	NT
LYS				
PHA	Positive	Positive	Positive	NT
ConA	Positive	Positive	Positive	NT
LIF				
PPD	Positive	Positive	Positive	NT
M. alb.	Positive	Positive	Positive	NT

[a] The PPD reaction was positive 2 years earlier.
[b] NT, not tested.
[c] N, normal range.

Acta Otolaryngol 82:212–215, 1976

IMMUNOLOGICAL FINDINGS IN A CASE OF COGAN'S SYNDROME

S. Edström and A. Vahlne

From the Department of Otolaryngology, Central Hospital, Mölndal, and the Department of Virology, Institute of Medical Microbiology, University of Göteborg, Göteborg, Sweden

Abstract. The immune system of a case of Cogan's syndrome was investigated. A transient depression of the cell-mediated immunity was found. Thus, during the acute stage of the disease, there was a depression of the cutaneous delayed hypersensitivity reaction to PPD and a decrease in the number of T cells. There were also signs of complement consumption. A possible pathogenesis based on immune complexes due to a preceding viral infection is discussed.

In 1945, David Cogan published four cases of nonsyphilitic interstitial keratitis and vestibulo-auditory symptoms (Cogan's syndrome). Since then, about 60 cases have been reported. The aetiology of the syndrome is unknown. However, in earlier reports (Albrite & Resnick, 1961, Fisher & Hellström, 1961), vascular alterations have been found corresponding to those in systemic disease. The purpose of the present investigation was to examine the immune system of a patient with Cogan's syndrome and evaluate the possibility of immune complex aetiology.

CASE HISTORY

A 15-year-old girl had in association with a common cold experienced the onset of tinnitus, dizziness and ocular pain. Two weeks later she noticed a hearing loss in her left ear and was admitted to hospital. The patient had previously been healthy. At the age of 7 she was BCG vaccinated and when tested six years later the PPD reaction (0.1 ml: 2 TU/ml) was positive.

Ophthalmological examination

The eye lids were moderately oedematous and the bulbs were red. The right corneal parenchyma showed a peripheral opaquefication and the corneal epithelium was diffusely oedematous on both sides. Media and eye grounds were normal. One week after admission to hospital the left corneal parenchyma was also opaquefied.

Otoneurological examination

The hearing threshold level fell on both sides (Fig. 1). The stapedius reflex test showed no elicitation at 120 dB. Glycerol test was negative. There was no nystagmus and the caloric test of vestibular function showed no reaction on either side.

Routine laboratory examinations

The sedimentation rate and white-cell count in blood were elevated. The polymorphonuclear percentage ranged from 58 to 77 per cent. Platelets were 550 000 per mm^3. Fibrinogen was elevated (6.63 g/l). Other routine laboratory investigations (including cytology of bone marrow, cytology and electrophoretic pattern of spinal fluid, function tests of heart, liver, thyroid and kidney, muscle biopsy). X-ray ex-

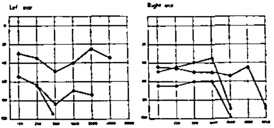


Fig 1 Hearing threshold levels during the first 6 days after admission to hospital ● admission ✱ 3 days after admission • 6 days after admission

amination of heart, lungs, ears porus acusticus and teeth) were normal

METHODS AND RESULTS

Lymphocyte function tests

In vivo tests of the cutaneous delayed hypersensitivity reaction to 0 1 ml PPD (2 TU/ml) were performed Lymphocyte stimulation tests were performed with the T cell mitogens Phytohaemagglutinin P (PHA) (Difco Laboratories, Detroit Michigan) and Concanavalin A (ConA) (Miles-Yeda Ltd, Rehovoth, Israel) as described by Mobacken & Lindholm (1974) Quantitation of T and B lymphocytes in venous blood was performed with the rosette formation test described by Jondal et al (1972) Inhibition tests of leucocyte migration in the presence of Monilia albicans and PPD extracts were performed using the polythene capillary tubing method of Hughes (1972)

When tested five weeks after the onset of the disease, there was no cutaneous delayed hypersensitivity reaction to PPD The patient was tested one year later, and at that time an indurated and reddened zone of 20 mm in diameter at the site of the PPD injection was noted after 48 hours (Table I) The percentage of T cells was subnormal during the first 6 weeks of the disease (Table I) No impairment of the qualitative in vitro T cell function was noted Thus the lymphocytes were normally stimulated with the mitogens tested and leucocyte migration was inhibited in the presence of PPD and *M albicans* (Table I)

Other immunological investigations

The complement factors C3 and C4 were determined with single radial immunodiffusion (Mancini et al , 1965), using Partigen immunodiffusion plates (Behringwerke AG, Marburg/Lahn, Germany) In the two first sera collected 3 and 6 weeks after onset of the disease subnormal values were obtained for C3

Table I *Lymphocyte function tests*

The cutaneous delayed hypersensitivity reaction to PPD (PPD) the percentage of T lymphocytes (T%) and B lymphocytes (B%) lymphocyte stimulation (LYS) with Phytohaemagglutinin (PHA) and Concanavalin A (ConA) leukocyte migration inhibition (LIF) in the presence of PPD and *Monilia albicans* (*M alb*)

Weeks after onset of the disease	5	6	11	56
In vivo test				
PPD[a]	Negative	NT[b]	NT	Positive
In vitro tests				
T% (N[c] 50–80%)	31%	32%	73%	NT
B% (N 10–20%)	12%	6%	23%	NT
LYS				
PHA	Positive	Positive	Positive	NT
ConA	Positive	Positive	Positive	NT
LIF				
PPD	Positive	Positive	Positive	NT
M alb	Positive	Positive	Positive	NT

[a] The PPD reaction was positive 2 years earlier
[b] NT not tested
[c] N normal range

Table II *Quantitation of the complement factors C3 (β1Cβ1A) and C4 (β1E)*

Weeks after onset of the disease	3	6	7	13
C3 (N[a] 140±30 mg%)	15 mg%	35 mg%	151 mg%	191 mg%
C4 (N 50–150%)	45%	70%	86%	>150%

[a] N normal range

(15 and 35 mg%, respectively, normal range 140±30 mg%) and in the first serum for C4 (45%, normal range 50–150%) (Table II)

Antinuclear antibodies in serum, assayed by immunofluorescence technique on tissue sections of rat liver and kidney, were not found IgM, IgG and IgA in serum determined according to Mancini et al (1965), were within normal limits

Virological investigations

As the patient had symptoms suggesting a viral infection in connection with the onset of the syndrome, efforts were made to establish an aetiological diagnosis of the infectious agent

Virus isolation attempts were performed by inoculating green monkey kidney cells, Hep 2 cells, human lung fibroblasts and primary rabbit kidney cells with test material from the nose throat conjunctival secretion and spinal fluid The cell cultures were visually examined by light microscopy daily for 10 days No cytopathic effect was noted with any of the material tested

A complement fixation (CF) method according to Sever (1962) with minor modifications was used for serological investigation Sera collected five days after admission to hospital and 4 weeks later were tested in parellel with antigens of adeno virus cytomegalo virus herpes simplex virus influenza A and B virus morbilli virus mumps virus polio 1 2 and 3 virus, rubella virus vaccinia virus coxsackie B virus, respiratory syncytial virus parainfluenza 1, 2, 3 and 4 virus and varicella zoster virus The sera were also tested with antigens of ornithosis, mycoplasma and toxoplasma gondii by the CF method Four units of antigen and two units of complement were employed No significant rise in the antibody titre to any of the antigens tested was detected

DISCUSSION

The aetiology of Cogan's syndrome is unknown However, vascular alterations have been found corresponding to those occurring in systemic disease (Albrite & Resnick, 1961, Fisher & Hellstrom, 1961) As SLE (Koffler et al , 1971) and polyartritis nodosa (Gocke et al , 1971) as well as stromal keratitis (Aronson & Moore, 1969) have been related to immune complex disease the immune system of the patient with Cogan's syndrome was investigated During the acute stage of the disease, alterations were found which could be compatible with a pathogenesis based on antigen–antibody complexes Thus, there were signs of complement consumption and a transient suppression of the cutaneous delayed hypersensitivity reaction to PPD and of the number of T cells Such an impairment of the cell mediated immunity could facilitate the generation of antigens in excess which are necessary for the development of circulating immune complexes (Mannik et al 1974) A depression of cell mediated immunity is encountered in a number of viral infections, such as measles (Starr & Berkovich 1964a) influenza (Kantzlee et al 1974) rubella (Olson et al 1968) acute infectious mononucleosis (Mangi et al 1974) chickenpox (Starr & Berkovich, 1964b), upper respiratory infections (Thomas et al , 1968) hepatitis B (Reisenbuk & Ioks 1974) and after vaccination with live attenuated mumps virus (Kupers et al , 1970)

The patient described in this report had an upper respiratory infection in association with the first symptoms of the disease Diagnosis of the infectious agent was not achieved However, this does not rule out a viral aetiology, as, for example, rhinoviruses would not have been diagnosed by the methods employed Other investigators have also reported upper respiratory infections in connection with Cogan's syndrome (Albrite & Resnick, 1961, Cogan, 1945)

Immune complex disease due to viral infections has previously been reported Thus, glomerulonephritis in experimental animals chronically infected with lactate dehydrogenase virus (Notkins et al , 1966), lymphocytic choriomeningitis virus (Oldstone & Dixon 1970) and Aleutian disease virus (Porter & Larsen, 1967), have been shown to be caused by circulating virus antibody complexes Lactate dehydrogenase virus (Michaelides & Schlesinger, 1974) and lymphocytic choriomeningitis virus (Hanaoka & Hotchin, 1969) have also been shown to cause a selective depression of the T cell response In man, Gocke et al (1971) found circulating immune complexes composing HB_sAG in patients with periarteritis nodosa

The mechanism by which immune complexes are deposited in the tissues and give injury is not known in every detail However immune complexes induce the clumping of platelets which with the participation of antigen sensitized basophiles release vasoactive amines The amines increase vascular permeability, and large complexes may be deposited in the tissues by a process of filtration (Cochrane & Koffler, 1973) Neutrophils may be attracted to the sites of immune complex deposition by complement mediated processes and release constituents capable of digesting and injuring the tissues (Henson 1971) In addition, immune complexes may activate components of the clotting system and cause the deposition of fibrin (Allison et al 1972)

The aural lesion of Cogan's syndrome might well originate from the cochlea (Djupesland et al , 1974, Serrins et al , 1963, Wolff et al 1965) Fisher & Hellström (1961) found mononuclear infiltrates in the cochlear spiral ligament and a dense coagulum in the cochlear duct and the semicircular canals According to our findings, it appears reasonable to assume that these derangements might be due to circulating or locally produced immune complexes deposited in the endolymphatic spaces leading to an impaired function of the sensory neural cells The new bone formation within the labyrinth reported by Wolff et al (1965) would be secondary to the inflammatory reaction (Kuntscher, 1967)

ZUSAMMENFASSUNG

Es wurde das Immunsystem eines Falles mit Cogan's Syndrom untersucht Eine vorübergehende Depression der zellgebundenen Immunität wurde gefunden Demgemäß war während der akuten Phase der Erkrankung eine Depression der cutanen Reaktion vom verzögernden Typ mit PPD und eine Verminderung der T Zellzahl vorhanden Auch fanden sich Zeichen von Komplementverbrauch Die Möglichkeit einer Pathogenese auf Grund von Immunovorgangen im Zusammenhang mit einer vorausgegangenen Virusinfektion wurde diskutiert

List of references may be requested from the authors

Acta Otolaryngol 82 216–218, 1976

CONCENTRATION OF IMMUNOGLOBULINS IN NASAL SECRETION FROM CHILDREN WITH RECURRENT INFECTIONS IN THE UPPER AIRWAYS

N Mygind and J -Å Wihl

From the Otopathological Laboratory University ENT Clinic Rigshospitalet Copenhagen Denmark and the University ENT Clinic Allmanna Sjukhuset Malmo Sweden

Abstract Concentrations of immunoglobulin G (IgG) IgA IgE and albumin were determined in nasal secretion from 14 children suffering from chronically recurrent infections in the upper airways and from normal subjects The only statistically significant difference observed was increased albumin values during infection

Constantly recurrent infections in the upper airways of children represent a challenge to the otorhinolaryngologist, both in clinical and in scientific work

For obvious reasons, attempts to discover an immunological etiology of the diseases have been made The results obtained by modern immunological methods have so far been disappointing as demonstrable immune deficiencies are found to account for only a very small part of the cases (Berdal et al , 1976) Obviously these findings do not exclude that we in the future by means of more advanced immunological methods will be able to discover immune deficiencies as etiological factors in a larger part of children with pronounced tendency for upper airway infections

In all cases of infection immune reactions are important factors which partly render the individual resistant to the micro organisms, and partly contribute to the inflammatory reactions and consequently to the symptoms Therefore, a detailed and exact description of the immune system during airway infection is required As we have no exhaustive knowledge of the local immune system of the mucous membrane during recurrent infections, we carried out an investigation of the quantities of immunoglobulin G (IgG), IgA, IgE and albumin in nasal secretion from children, representing particularly severe cases of constantly recurrent infections in the upper airways

PATIENTS AND METHODS

A total of 14 patients were included in the study Their average age was 3 4 years (range 2–11 years) They represent some of the most troublesome cases with recurrent upper air way infections, seen at the ENT clinic Allmanna Sjukhuset, Malmo This ENT clinic is the only one available for about 200 000 subjects

In all patients, nasal secretion was collected both in the acute infectious state and in a symptom free period As control subjects were used 10 children of the same age admitted to the clinic for other diseases which were not infectious, allergic or involved the airways All samples were collected betwee 10 a m and 2 p m

Secretion was obtained by filter pape placed in the nasal cavity As the filter pape was weighed before and after it was placed i the nose, results can be given in absolute va ues IgG, IgA and albumin were determine by a Mancini radial immunodiffusion tech nique and IgE by radio immuno-sorbent tech nique Human serum was used as a standard

Table I *Protein concentrations in nasal secretion of normal children and children suffering from chronically recurrent infections in the upper airways*

		No of subjects	Median (mg/100 ml)	25–75 percentile (mg/100 ml)	Mann Whitney rank sum test
Albumin	Patients infected period	14	220	108–300 } $p<0.05$	} $p<0.01$
	Patients non infected period	10	61	37–116	
	Normal children	10	50	27– 80	
IgG	Patients infected period	14	34	19–44	
	Patients non infected period	10	30	17–64	No significant differences
	Normal children	10	17	11–39	
IgA	Patients infected period	14	26[a]	19–49[a]	
	Patients, non infected period	10	20	17–46	No significant differences
	Normal children	10	22	15–33	
IgE	Patients infected period	14	6[b]	<5–8	
	Patients non infected period	10	(5)	<5–7	No significant differences
	Normal children	10	(5)	<5–7	

[a] As serum IgA is used as a standard for determination of secretion IgA it is most correct to multiply the actual IgA values by a factor of 2 5
[b] All IgE values are Units/ml

also for IgA All determinations were carried out on clear, sterile fluid passed through a Millipore filter The methods are described in greater detail elsewhere (Mygind et al , 1975)

RESULTS

Data are few and the scatter of the single de terminations high Therefore, the results are given as median and percentiles Indications of mean and standard deviation would be incorrect and misleading From Table I is seen that the only significant difference was elevated albumin values in the patients during infection IgG was slightly and insignificantly raised in the patients, both in infected and in non infected periods No differences were found for IgA and IgE

The collection method worked satisfactorily in most children As the procedure produced weeping in some of the children, the nasal secretion was contaminated with tears

For the low IgE levels no exact values can be given, as the determinations are rather un certain the border of the method's sensitivity Due to dilution with saline, results of analyses had to be multiplied by a factor of 2–5

DISCUSSION

In the children with recurrent upper-airway infections the average albumin concentration during infection was 4½ times higher than the normal average value, due to vascular leakage of serum proteins When the patients were without signs and symptoms of infection the albumin level was normal

The slight and insignificant increase of IgG during infection persisted unchanged during clinical non-infectious periods Therefore it is reasonable to suggest that these patients also in symptom free periods, have an increased local IgG production, like that found in patients suffering from chronic purulent rhinitis (Brandtzaeg, 1974)

In most cases the amounts of IgE determined were so low as to be non-detectable or borderline values for what is detectable One of the "normals" had a significantly raised value (80 U/ml) without any symptoms of allergy It has previously been demonstrated that patients suffering from hay fever and especially nasal polyps often have raised IgE values in nasal secretion (≥15 U/ml) (Mygind et al , 1975) The low IgE level found in infected children demonstrates that the high

IgE values in hay fever and nasal polyposis are not merely due to hypersecretion and inflammation

We found no differences in the concentrations of IgA, between patients with recurrent upper-airway infections and normals, or between infected and non infected states Other investigators have suggested that patients with low IgA concentration in nasal secretion are more susceptible to upper-airway infections than those with high IgA values (Rossen et al, 1970, McCormick et al, 1972) It has also been found in some investigations that the IgA concentration increases during acute infections (Cohen et al, 1970, Butler et al, 1970)

The interpretation of these conflicting data is difficult and may involve methodological considerations It is a source of errors in previous studies, using nasal washings, that exact absolute values cannot be given—an exception is the method used by Cohen and collaborators When the results are given as mg per ml of lavage fluid an elevated IgA value may as well be due to a high IgA concentration in secretion as to a large amount of secretion, obtained by nasal lavage This source of uncertainty is eliminated in the paperstrip method actually used, but also this method has shortcomings, as it stimulates nasal and lacrimal secretion While the admixture of lacrimal secretion, with another immunoglobulin pattern is undesirable it is possibly an advantage that the nasal secretion is stimulated By protein analyses of saliva it has been shown that a stimulation of the secretion gives the most reproducible results (Oon & Lee, 1972)

In conclusion it may be said that the present investigation disclosed no changes in nasal IgA concentration during infection but further studies are required especially as regards the methods used for collection of secretion In all quantitative analyses of nasal secretion it must be required that the concentrations are given in absolute values (mg/100 ml of secretion)

ACKNOWLEDGEMENTS

Thanks are due to Dr Bent Weeke Laboratory for Clinical Allergy Rigshospitalet Copenhagen for determinations of IgE

ZUSAMMENFASSUNG

Bei 14 Kindern mit chronisch rezidivierenden Infektionen der oberen Luftwege wurden die Konzentrationen von Immunoglobulin G (IgG) IgA IgE und Albumin in Nasensekret bestimmt Als einziger statistisch signifikanter Unterschied wurde eine Erhohung der Albuminwerte wahrend der Infektion gefunden

REFERENCES

Berdal P Brandtzæg P Frøland S S Henriksen S D & Skrede S 1976 Immunodeficiency syndromes with otorhinolaryngological manifestations *Acta Otolaryngol* (Stockh) *82*, 185

Brandtzæg P 1974 Mucosal and glandular distribution of immunoglobulin components *Immunology 26* 1101

Butler W T Waldmann T A Rossen R D Douglas R G & Conch R H 1970 Changes in IgA and IgG concentrations in nasal secretions prior to the appearance of antibody during viral respiratory infection in man *J Immunol 105* 584

Cohen A B Goldberg S & London R L 1970 Immunoglobulins in nasal secretions of infants *Clin Exp Immunol 6* 753

McCormick D P Wenzel R P Davies J A & Beam W E 1972 Nasal secretion protein responses in patients with wild type adenovirus disease *Infect Immunol 6* 282

Mygind N Weeke B & Ullman S 1975 Quantitative determination of immunoglobulins in nasal secretion *Int Archs Allergy Appl Immunol 49* 99

Oon C H & Lee J 1972 A controlled quantitative study of parotid salivary secretory IgA globulin in normal adults *J Immun Methods 2* 45

Rossen R D Butler W T, Waldman R H, Alford R H Hornick R B Togo Y & Kasel J A 1970 The proteins in nasal secretion *J Am Med Ass 211* 1157

Acta Otolaryngol 82 219–221, 1976

DIURNAL VARIATION OF NASAL PROTEIN CONCENTRATION

N Mygind and J Thomsen

From the Otopathological Laboratory University ENT Clinic Rigshospitalet Copenhagen Denmark

Abstract In normal subjects a marked diurnal variation was disclosed in the concentrations of immunoglobulin G (IgG) IgA and albumin in nasal secretion the night values being 4 5–5 5 times higher than those during the day It is suggested that this is due to changing secretory activity of the nasal glands

Measurement of proteins in nasal secretion gives results far more variable than the results of corresponding serum determinations While normal limits for immunoglobulin G (IgG) in serum is 800–1 680 mg/100 ml we found a range of 6–80 mg/100 ml in nasal secretion of normal subjects (Mygind et al , 1975) This wide range reduces the value of protein de terminations in nasal secretion It is also a serious disadvantage that no generally ac cepted and standardized method for the col lection of nasal secretion exists In addition concentrations of the individual proteins are usually given as percentages of total protein, and not in absolute values per ml of secretion

Therefore, attempts are required for ex plaining the considerable scatter of results and for standardizing the collection methods A valuable contribution was made by Hughes & Johnson (1973), who disclosed a marked diurnal variation in nasal IgA concentration

The purpose of the investigation reported here was twofold (1) to repeat the study of Hughes & Johnson with a collection method which gives the results in absolute values and (2) to extend the diurnal studies to IgG and albumin

PATIENTS AND METHODS

Ten hospitalized patients from the ENT Clinic participated in the investigation None of them had diseases involving the upper respiratory tract and none had a macroscopically abnormal nasal mucosa All had a negative history of allergic diseases and recurrent infections in the airways During one day nasal secretion was collected at the following hours 08, 12, 16, 20, 24 and 04

To obtain nasal secretion two strips of fil ter paper (50×18 mm) were used The paper strips were weighed together with a small plastic tube and a stopper After filling the tube with 0 8 ml of a solution containing 0 9% saline and 0 1% sodium acid a new weight was obtained By use of nasal speculum the filter paper was then placed in the nasal cavity for 15 minutes After removal, the paper was placed in the saline and stored at −20°C On the day of analysis the plastic tube, the stopper, the eluent fluid and the secretion was weighed again The amount of secretion was calculated to be from 100 to 500 mg and the factor of dilution from 2 5 to 9 The solution was passed through a Millipore filter (pore size 0 2 μm), resulting in about 0 6 ml of a clear and sterile fluid The sample was dis carded, if Hemastix paper strips showed con tamination with blood

Albumin, IgG and IgA were determined by radial immunodiffusion according to Mancini Low concentration plates were used (Behring

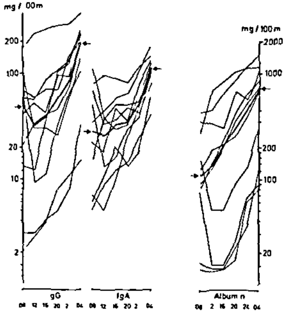


Fig 1 Diurnal variation of immunoglobulin G (IgG) IgA and albumin concentrations in nasal secretions from 10 normal subjects Average values (median values) are indicated (→—←)

werke AG, Marburg) Serum immunoglobulins were used as standards for all measured immunoglobulins including IgA As serum IgA is monomer and IgA in secretion mainly secretory IgA (dimer plus secretory component), the procedure underestimates the IgA concentration by a factor of 2–3

RESULTS

All data are given in Fig 1 In every patient there was a characteristic circadian pattern for all three proteins investigated The values increased somewhat during the daytime, and considerably during the night when the patients were asleep During the morning concentrations decreased rapidly Therefore the period between 08 and 12 a m was the only time of the day where concentrations could both decrease and increase

The average (median) concentrations during the daytime (12 a m and 4 p m) were 35 mg/100 ml IgG, 30[1] mg/100 ml IgA and 170 mg/100 ml albumin while the corresponding values during night (midnight and 04 a m) were 140, 80[1] and 590 All differences were highly significant (Wilcoxon s sign rank test $p<0.001$) This implies that the levels of nasal IgG, IgA and albumin are 3–4 times higher during night than during daytime

Fig 1 clearly shows that there are marked interindividual differences which are constant during the day Apart from the early morning values, the determinations in the individual patients vary during the 24 hours according to a predictable pattern running parallel to the average diurnal figure

It is hereby demonstrated that marked interindividual differences and a characteristic diurnal pattern account for a major part of the very wide range within which the total number of determinations varies

DISCUSSION

Hughes & Johnson (1973) found a marked diurnal variation of IgA concentration in nasal secretion with low concentrations during the daytime and high concentrations during the night These authors explained the observation as being due to variation in local IgA production Even though the present study disclosed almost the same diurnal pattern it also indicates that the varying IgA concentration is more likely to be due to a changing amount of secretion produced

For explaining this statement a discription of the pathways of IgA, IgG and albumin in the nasal secretion is necessary Dimer IgA is synthesized in periglandular plasma cells and actively transported through the glandular epithelium appearing in the secretion as secretory IgA IgG is partly synthesized by plasma cells in the lamina propria and partly a transudate product from the blood IgG in the tissue fluid passes through the surface epithelium to the secretion Albumin is not synthesized in

[1] As a serum IgA standard was used the values for IgA in secretion have to be multiplied by a factor of 2 5

the nasal mucosa and all albumin in the secretion is a transudate product from the blood

In the present study, identical diurnal variations were found for all proteins investigated If differences in the production rate should account for the diurnal variation—as claimed by Hughes & Johnson—parallel changes should occur in quite different processes such as glandular 'secretion' of secretory IgA, local IgG production in the mucosa and leakage of plasma proteins through blood vessels and epithelium, which is unlikely

A large contribution of mucus to the secretion may reduce the concentration of IgA, IgG and albumin, by adding a volume deficient in these proteins to the total amount of secretion If the production of serous secretion is influenced by the same mechanisms as production of saliva (Brandtzæg, 1971), a high secretory activity is followed by a low immunoglobulin concentration Consequently changes during the day in the secretory rate of mucous and serous secretions may very well account for the observed diurnal variation in nasal secretion proteins As is most evident in patients with perennial rhinitis, but also in normal subjects the secretory activity is pronounced during the morning while the nasal mucosa is drier in the night Particularly at 04 a m , it was rather difficult to collect a sufficient amount of secretion for analysis

In conclusion nasal secretion for protein determination should be collected during a fixed period of time in the day (e g 10 a m – 2 p m) and never in the early morning As the diurnal variation is supposed to be a consequence of changing secretory activity, no constant diurnal variation is expected in rhinitis patients, with changing degrees of nasal hypersecretion

It seems most reasonable to explain the observed diurnal variation in IgG, IgA and albumin as a consequence of changing secretory activity In our opinion, there is no evidence to suggest a diurnal variation in the production rate in the nasal mucosa

ZUSAMMENFASSUNG

Bei normalen Versuchspersonen wurden bedeutende tagliche Variationen von den Immunoglobulin-G-(IgG)- IgA und Albuminwerten im Nasensekret gefunden Die Nachtwerte zeigten sich 4,5–5 5mal hoher als die Tageswerte Als Ursache denken wir uns Anderungen in der sekretorischen Aktivitat der Nasendrusen

REFERENCES

Brandtzæg P 1971 Human secretory immunoglobulins VII *Archs Oral Biol 16* 1295

Hughes E C & Johnson R L 1973 Circadian and interpersonal variability of IgA in nasal secretions *Ann Otol Rhinol Laryngol 82* 216

Mygind N Weeke B & Ullman S 1975 Quantitative determination of immunoglobulins in nasal secretion *Int Archs Allergy Appl Immunol 49* 99

Acta Otolaryngol 82 222-225, 1976

A NEW ANTI-ALLERGIC DRUG ICI 74 917 AEROSOL

J Thomsen and N Mygind

From the Otopathological Laboratory Rigshospitalet Copenhagen Denmark

Abstract Ten hay fever patients were challenged intra nasally with allergen before half an hour 2 hours and 4 hours after intranasal use of disodium cromoglycate powder placebo and ICI 74 917 a new aerosoled com pound with anti allergic action The new compound showed a maximum protective effect after half an hour and a significant difference was found also after 2 hours The effect was also significantly better than that of di sodium cromoglycate powder The results were positive enough to make further human studies of ICI 74 917 desirable

One of the most significant advances to date in the management of allergic diseases in the airways was the discovery of disodium cromo glycate (DSCG) Discovered by Altonyan (1967) and introduced in 1968, DSCG has both made an important contribution to our under standing of some aspects of antigen antibody interactions and has come to be recognized as a valuable agent in the treatment of some types of asthma and rhinitis However, in the treatment DSCG has some shortcomings (1) It does not act in all patients suffering from allergic asthma and rhinitis (Brogden et al, 1974) (2) As the single dose is large, the drug cannot be given in an aerosol but has to be given as a powder (3) The duration of the maximum protective effect is short making a continuous protection of the patients during the day and particularly during the night dif ficult to maintain (Georg et al 1973, Brogden et al, 1974)

Therefore several attempts have been made in pharmaceutical companies to develop new and better drugs with the same mode of action as DSCG, i e stabilizing the mast cells and thereby inhibiting the immune determined de granulation and liberation of histamine and slow-reacting substance

ICI 74 917 is a phenanthroline with anti allergic properties Administered intravenous ly to rats at the same time as antigenic chal lenge, it inhibited passive cutaneous anaphylaxis It was 300 times more active than DSCG in this model (Evans et al, 1974) As experiments showed that the compound was not an antagonist to histamine or 5 hydroxytryptamine, these findings suggest that ICI 74 917 is an inhibitor of mediator release induced by IgE like antibodies (Evans & Thomson, 1975)

On the basis of its anti allergic properties and lack of toxicity in animal studies there was good reason to investigate whether this compound is active in the prophylaxis of allergic airway diseases in man

The purpose of the actual trial was to elucidate three main problems (1) Is the compound active in man? (2) What is the degree of efficacy as compared with DSCG? (3) How long is the duration of the protective effect? To solve these problems serial intranasal allergen challenges in hay fever patients was a method found suitable for the purpose

Ten young persons suffering from grass-pollen hay fever were studied in February–April, i e outside the grass pollen season Each person was exposed to serial intranasal

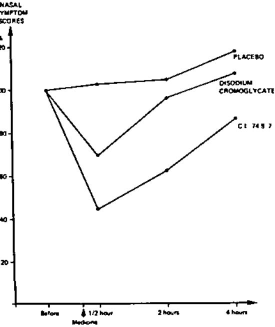


Fig 1 Mean nasal symptom scores by intranasal allergen challenges before half an hour 2 hours and 4 hours after intranasal use of the drug or placebo The pre treatment value in the individual patient constitutes 100 %

allergen challenges on three occasions at intervals of at least one week The challenges were carried out with the same allergen dosage each time before half an hour 2 hours and 4 hours after the administration of the drug The three treatments given in random order to each test person were (1) 0 5 mg ICI 74 917 into each nostril delivered from an aerosol canister equipped with a special nasal adaptor (2) One puff into each nostril of an identical placebo aerosol (3) 10 mg DSCG powder into each nostril delivered from the commercially available Lomudal Nasal® applicator None of the test persons had any experience with DSCG treatment beforehand Before each challenge series they were informed that the aerosol/powder might be active or placebo As the investigators knew the property of the powder the trial was only double blind as regards the aerosol

As is known from diagnostic clinical work intranasal allergen challenge is connected with some problems First large allergen quantities are necessary Secondly, the symptoms are difficult to quantify in an objective and reliable way The parameters used for scientific work are measurements of nasal airway resistance by rhinomanometry This method was supposed to be less suitable for the actual purpose, serial challenges Therefore we decided to measure the two other rhinitis symptoms, sneezing and rhinorrhoea After some pilot studies the following procedure was found simple and sufficiently reliable for our purpose Immediately after the challenge the test person assumed a "praying" position the head bent forward and the hands folded For a period of 15 minutes the subject counted and recorded the number of sneezes and drips from the nose These were added together to give the "sneeze-drip" score

In a pretrial session the allergen dosage for each subject was determined A sneeze drip score between 15 and 30 was found suitable In three subjects 2000–6000 grass pollen grains were applied intranasally by means of a rubber balloon and a capillary tube In seven 0 25 ml of a dialysed allergen extract was sprayed intranasally The concentration varied from 1/20 to 1/50 w/v In all cases the allergen was timothy grass

The first challenge was carried out between 8 and 9 a m When the nose was again completely normal, as checked by rhinoscopy usually after one hour, the treatment was given The subject was challenged half an hour 2 hours and 4 hours after the treatment was applied to the nasal mucosa Each time the nostril with the best air flow was chosen for challenge

RESULTS

ICI 74 917 was shown to have a partially protective effect on the allergen-challenged nasal mucosa Half an hour after use the drug showed maximum effect as the symptom scores were significantly lower than after use of placebo ($p<0.01$, Wilcoxon's sign rank test) This was also the case after 2 hours The

effect decreased, and although there was a tendency, no significant difference was found after 4 hours (Fig 1)

Significantly lower scores were found with ICI 74 917 after half an hour and 2 hours, as compared with DSCG ($p<0.05$) After 4 hours a tendency, but no significant difference, was found After placebo treatment there was a slight, but insignificantly higher symptom score after each challenge Local hyposensitization was no problem in obtaining a proper hay fever attack even though these patients were challenged more than 12 times over a period of 1–12 months

Four serial challenges a day was the maximum After this, most patients had nasal symptoms for some days These troubles were alleviated by giving the patients xylometazoline (Otrivin®) and beclomethasone dipropionate aerosol (Beconase®) for 2 days after each challenge series In all cases, at least one week was allowed to pass between the end of one treatment and the start of the next challenge series

DISCUSSION

The trial showed that ICI 74 917 has a protective effect on the allergen challenged human respiratory mucosa In the trial, the effect was superior to that of DSCG This may be due to the distribution of the aerosoled ICI 74 917, which is more similar to that of the sprayed allergen than the distribution of the DSCG powder, which as judged by rhinoscopy, is poor The major part of the powder is visible in the vestibule and the anterior third of the nasal cavity immediately on a proper delivery procedure As ICI 74 917 was found 300 times more active in the rat passive cutaneous anaphylaxis system it is also possible that ICI 74 917 is the most active drug in human challenge situations The fact that the degree of DSCG protection in our trial was less than that found in other intranasal challenges (Engstrom, 1971, One et al 1970, Taylor & Shivalkar, 1971) is probably due to the very large allergen dosage used in this trial This may possibly also explain why we—unlike Taylor & Shivalkar (1972)—did not find a local hyposensitization after several challenges

One of the major shortcomings in the DSCG treatment of allergic diseases of the airways is the relatively short duration of the maximum protective effect, making it difficult to obtain a continuous protection, particularly during the night (Georg et al, 1973) The present study indicates that ICI 74 917 is superior in this respect, even though exact conclusions as to the duration of action of the drug cannot be drawn from our results

It may very well be an advance in the treatment of allergic diseases, especially in the lower airways, that the active dose is less than 2 mg so that it can be delivered by a pressurized aerosol, which probably gives a better distribution on the mucosa than the powder delivery systems An aerosol is also more readily accepted by the patients, and a more frequent use may be tolerated However, these statements have to be confirmed by clinical trials in which allergens are presented to the mucous membrane in a more "physiological" way In conclusion the result of our trial shows that ICI 74 917 is active, and further human trials are therefore desirable

The method used was very simple and suited for the purpose, but not totally satisfactory as the allergen doses had to be very high, and the scores varied considerably from time to time A combination of the sneeze drip score and measurements of airway resistance by rhinomanometry is supposed to be the method of choice

ACKNOWLEDGEMENT

Our thanks are due to Imperial Chemical Industries Limited U K for the supply of aerosols

ZUSAMMENFASSUNG

Zehn Heuschnupfenpatienten wurden mit Allergen nasal provoziert Dies geschah vor eine halbe Stunde zwei

Stunden und vier Stunden nach nasaler Anwendung von Dinatriumcromoglycat Pulver einem unwirksamen Mittel und ICI 74 917 einer neuen arosolen Zusammensetzung mit antiallergischer Wirkung Das neue Heilmittel zeigte ein Maximum an schutzender Wirkung nach einer halben Stunde und ein signifikanter Unterschied wurde auch nach zwei Stunden festgestellt Die Wirkung war auch bedeutend besser als die von Dinatriumcromoglycat Pulver Die Resultate waren positiv genug um weitere menschliche Versuche wunschenswert zu machen

Reference list can be required from the authors

Acta Otolaryngol 82 226–229, 1976

GENETIC ASPECTS OF SOME OROFACIAL ANOMALIES

E Dahl

From the Department of Orthodontics Royal Dental College Copenhagen Denmark

Abstract The increased importance of congenital anomalies in childhood disease is stressed It is suggested that treatment and research in congenital craniofacial anomalies would benefit from centralized registration Their clinical management should be delegated to centres with a multidisciplinary team Some problems related to genetic counselling and identification of microforms are illustrated by cleft lip and palate An account is given of some orofacial anomalies

The development of the dentition and the craniofacial structures is considerably influenced by genetic factors, and many anomalies in this region display a hereditary pattern

The conquest of nutritional and infectious diseases in infants in the developed countries have exposed the congenital anomalies as a major factor in childhood disease (Warkany, 1971)

The exact prevalence of congenital craniofacial anomalies is unknown Only for cleft lip and palate are reliable incidence figures available From the centralized recording of cleft deformities in Denmark the prevalence at birth is estimated to be 2 per thousand (Fogh Andersen 1966) According to this approximately 600 children with facial clefts will be born annually in Scandinavia A centralized recording of other congenital craniofacial anomalies would highly facilitate the delineation of the clinical problems and their appropriate management in these patients and would render it possible to observe variations in prevalence

Congenital anomalies of the craniofacial structures affect some of man s most important sources of communication the facial appearance and the organs of speech hearing and vision They represent a considerable medical and social problem

Some congenital craniofacial anomalies are caused by a genotype which expresses itself in virtually all environments, others by chromosome aberrations and a few are caused by non recurrent environmental damage The majority, however, is multifactorial, i e a combined action of deviations in several gene positions and environmental factors Even within one clinical category, such as facial clefts, some cases may have a major genetic component, and other cases clinically indistinguishable from them, may have a major environmental cause Therefore no classification of the anomalies by their heredity is attempted

Clinical management of craniofacial anomalies often presents complex problems which involve several professional disciplines This is exemplified by the service given to cleft lip and palate patients who need care from the specialties of plastic surgery dentistry, oto-rhinolaryngology and speech therapy Experience has shown that optimal rehabilitation is best obtained in centres where the number of patients is sufficient to establish comprehensive clinical experience and reliable data for research It is suggested that treatment and research in other craniofacial anomalies would benefit from a similar integrated multidisciplinary approach

The final aim of the study of congenital anomalies is prevention To this end, aetiological factors must be found and their inter action explained In most congenital disorders, the aetiology is unknown, and at present the most obvious prophylactic possibilities still seem to be the control of environmental teratogenic factors and genetic coun selling Some principal problems in genetic counselling can be illustrated by cleft lip and palate Fogh Andersen (1942) was able to show that cleft lip with or without cleft palate is genetically different from isolated cleft palate The former is found more often in males and the latter more frequently in fe males Only occasionally do the two types occur in the same family, and the risk of recurrence is somewhat different

The common types of clefts are compatible with a multifactorial threshold model (Fraser, 1970) If one parent is affected, the risk for the first child is 3–5% If the cleft is part of a syndrome, the mode of inheritance is often quite different Over 100 syndromes are known (Gorlin et al , 1971, Gorlin & Cervenka, 1974) that involve clefts of the lip or palate About one third of them are caused by major mutant genes As an example may be mentioned the combination of cleft lip and palate and paramedian fistulae of the lower lip, which is transmitted as an autosomal dominant trait This shows that accurate delineation of separate entities within categories can improve the quality of genetic coun selling A corresponding subdivision may be possible in a number of the classical syn dromes

Another genetic approach is concerned with identifying those individuals in a population who carry a gene for a given affection, but who do not clearly demonstrate the phenotype for that gene This implies that accurate definition of the phenotype is most important In connection with cleft lip and palate a num ber of structural deviations related to the cleft region have been suggested as microforms of the cleft which should occur more frequently in close relatives of persons with the defect than in the general population This appears to be true for cleft uvula and submucous cleft palate, whereas it is questionable for nasal asymmetry and does not seem to be true for missing maxillary lateral incisors Roentgen-cephalometric studies show that clefts of the secondary palate are often accompanied by characteristic changes in facial shape and that differences in this respect exist between different types of cleft (Dahl, 1970) Further studies are needed to reveal if such traits can discriminate genetic susceptibility

An account of some genetically determined orofacial anomalies is given below

Amelogenesis imperfecta, in which the tooth enamel exhibits varying types of defect, is an example of a hereditary anomaly localized to the dentition *Dentinogenesis imperfecta* is inherited as an autosomal dominant trait with variable expressivity, but with a high degree of penetrance It occurs as an independent trait or as part of the osteogenesis imperfecta syndrome

Most cases of *hypodontia* (congenital absence of some of the teeth) are probably determined by genetic factors The condition is generally a local one, but may be one manifestation of a generalized ectodermal defect

Cleidocranial dysostosis is inherited as an autosomal dominant trait although spontaneous cases are quite common The anomaly presents itself as an ossification defect, espe cially although not exclusively, of the membranous bones and mainly localized in the midline The patients are usually short The head is brachycephalic with frontal and parietal bossing Closure of fontanelles and sutures is delayed and the lack of fusion of the frontal bones may extend into the nasal process resulting in a depression of the root of the nose Wormian bones are formed in the suture lines The mandibular symphysis may remain open The oral manifestations include supernumerary teeth, and tooth eruption is de layed or absent Submucous cleft palate may occur. There is aplasia or hypoplasia of the

clavicles, which permits a remarkable range of shoulder movement Other skeletal deviations are reported, e g of the pelvic bones

Premature closure of sutures in the neurocranium and facial skeleton is the cause of a series of craniofacial anomalies If localized in the neurocranium, they result in morphological changes, e g *oxycephaly* when the coronal suture is synostosed and *scaphocephaly* when the sagittal suture is prematurely closed These simple cranial synostoses usually exhibit no genetic transmission But in these cases, too, the combination with other anomalies can alter the pattern of inheritance Thus, the combination of oxycephaly and syndactyly between the 2nd and 3rd fingers (Saethre-Chotzen syndrome) exhibits autosomal dominant inheritance (Kreiborg et al , 1972) In *craniofacial dysostosis* (Crouzon syndrome), premature synostosis occurs in calvarian sutures, possibly in sutures of the cranial base, and in maxillary sutures An autosomal dominant inheritance has been shown The craniofacial growth is considerably disturbed The synostosis may be progressive, and the deviations aggravate during growth The head is brachycephalic, marked digital impressions are seen in radiographs of the skull, the sella turcica is large and the orbits are shallow, which contributes to the characteristic exophthalmos The arrest of growth of the midfacial sutures results in an increasing disparity in the facial skeleton Secondary involvement of the optic nerve and other neurological symptoms can be found

Otocraniofacial syndromes or first and second visceral arch anomalies include a wide spectrum of constellations of symptoms Their proper delineation developmental relations and causal mechanisms are still obscure *Mandibulofacial dysostosis* (Treacher Collin syndrome) is inherited as an autosomal dominant trait with variable expressivity and incomplete penetrance, but sporadic cases are not uncommon The major features include hypoplasia of the malar bones, antimongoloid slant of palpebral fissures coloboma of the lower lid, and dysplasia of the mandible Ear anomalies, macrostomia and cleft palate may occur The soft tissues are often poorly developed The combination of symptoms and the degree of their manifestation may vary considerably In its full expression the skeletal deviations include the sphenoid and the temporal bones (Poswillo, 1973, Dahl et al , 1975), which are not developed from the first or second branchial arch The anomaly has been explained as a regional, embryological vascular defect The characteristic facial shape is maintained relatively unchanged during growth

Hemifacial microsomia and Goldenhar syndrome are examples of unilateral otocraniofacial anomalies They do not seem to show genetic transmission, and the symptoms are somewhat different from mandibulofacial dysostosis, e g there is often larger involvement of the outer ear

ZUSAMMENFASSUNG

Die steigernde Bedeutung von aneeborenen Anomalien wird betont Zentralisierte Registrierung ist fur die Behandlung und Forschung der angeborenen craniofacialen Anomalien sehr bedeutungsvoll Die klinische Behandlung von diesen Anomalien sollte in Zentren mit einem multidisziplinaren Team stattfinden Einige Probleme der genetischen Beratung und der Identifizierung von Mikroformen werden durch Lippen Kiefer Gaumenspalte illustriert Einige otofaciale Anomalien werden besprochen

REFERENCES

Dahl E 1970 Craniofacial morphology in congenit[illegible] clefts of the lip and palate *Acta Odont Scand 2[illegible]* Suppl *57*

Dahl E Kreiborg S & Bjork A 1975 A morpho[illegible] logic description of a dry skull with mandibulofac[illegible] dysostosis *Scand J Dent Res 83* 257

Fogh Andersen P 1942 *Inheritance of harelip and clef[illegible] palate* Nyt Nordisk Forlag Copenhagen

— 1966 Incidence and etiology of clefts of lip alveolu[illegible] and palate in humans In Schuchardt K (ed) *Treatment of patients with clefts of lip alveolus and palat[illegible]* pp 4–8 Georg Thieme Verlag Stuttgart

Fraser F C 1970 The genetics of cleft lip and cleft palate *Am J Hum Genet 22* 336
Gorlin R J & Cervenka J 1974 Syndromes of facial clefting *Scand J Plast Reconstr Surg 8* 13
Gorlin R J Cervenka J & Pruzansky S 1971 Facial clefting and its syndromes *Birth Defects Orig Art Ser 7* 3
Kreiborg S Pruzansky S & Pashyan H 1972 The Saethre-Chotzen syndrome *Teratology 6* 287
Poswillo D 1973 The pathogenesis of the first and second branchial arch syndrome *Oral Surg 35* 302
Warkany J 1971 *Congenital malformations* Year Book Medical Publishers Chicago

Acta Otolaryngol 82 230–233, 1976

HEREDITY AND SYMPTOMS IN ACROMEGALY

J E G Kinnman

From the Department of Otolaryngology Karolinska sjukhuset and King Gustaf V Research Institute Stockholm Sweden

Abstract The author has performed clinical and follow up studies of 80 acromegalic patients treated by trans anthro-sphenoidal removal of the pituitary adenoma Heredity for acromegaly or gigantism was recorded in 3 8% of the patients and other hereditary factors in 13 8% Head trauma meningitis or encephalitis was recorded in the case histories in 18 8% The predominant symptoms were sweating paraesthesiae headache and joint pain Acromegaly was in 37 6% associated with goitre parathyroid adenoma gastric or duodenal ulcer parotid tumours or submandibular swelling The fecundity among the married patients was good 34 4% having three or more children Successful pregnancies occurred after the transanthro-sphenoidal removal of the adenoma

Acromegaly is a disease which, untreated, leads to significant disability and shortening of life expectancy through intracranial extension of the tumour diabetes mellitus, hypermetabolism splanchnomegaly and ultimately heart failure The life expectancy of acromegalic patients is shorter than that of patients suffering from non functioning chromophobic" adenoma (Bakay 1950) Deaths in acromegalic patients is more often due to secondary effects of STH (somatotropic hormone) overproduction than to local effects of the tumour (Henderson 1938)

Davidoff (1926) stated that hereditary influences on the disease exist although only 4 of the 100 patients in his series had a definite family history of acromegaly

A frequent early symptom of acromegaly is amenorrhoea in women and impotence and/or decreased libido in men (Marie, 1886 1890, Cushing 1909) Davidoff (1926) in his series of 100 patients with acromegaly, observed that the fecundity was below the usual average, and the number of children born to those who married after the onset of the disease was small

CASE MATERIAL

During the period 1961–1972, 47 male and 33 female patients with acromegaly were treated with transanthro sphenoidal surgery for removal of the pituitary adenoma At operation the majority of patients (approx 62%) were in the age group from 41 to 60 years The average age at onset of the disease was estimated to be 34 8 years

Only in three (male) patients did the family history reveal that several members were of high stature Diabetes mellitus occurred among relatives in five patients goitre in three, gastric ulcer in one, epilepsy in one and osteogenesis imperfecta with otosclerosis in one (Table I)

In four male patients, primary hyperparathyroidism had been established—goitre had been diagnosed in two of these patients, and one of them also suffered from duodenal ulcer and otosclerosis One of the patients in this group had a pronounced submandibular gland swelling and marked dryness of the mouth

One patient had been operated on for a carpal tunnel syndrome 5 years prior to the

Table I *Hereditary factors, previous diseases and fecundity in 80 acromegalic patients*

Values within parentheses are percentages

	Males	Females	Total
Hered for acromeg or gigantism	3 (6 4)	0	3 (3 8)
Other hered factors	7 (14 9)	4 (12 1)	11 (13 8)
Head trauma	7 (14 9)	3 (9 1)	10 (12 5)
Meningitis	0	2 (6 1)	2 (2 5)
Encephalitis	1	2 (6 1)	3 (3 8)
Goitre	4 (8 5)	9 (27 3)	13 (16 3)
Parathyr adenoma	4 (8 5)	0	4 (5)
Gastric or duodenal ulcer	6 (12 8)	2 (6 1)	8 (10)
Varicose veins	1	3 (9 1)	4 (5)
Haemorrhoids	6 (12 8)	1	7 (8 8)
Parotid tumour	1	1	2 (2 5)
Swelling of submandibular gland	2 (4 3)	1	3 (3 8)
Hearing dist	3 (6 4)	0	3 (3 8)
Hernia	9 (19 1)	1	10 (12 5)
Arnold Chiari anomaly	0	2 (6 1)	2 (2 5)
Epilepsy	0	2 (6 1)	2 (2 5)
Fecundity among married patients (n=61)			
No children	4 (11 1)	6 (24 0)	10 (16 4)
One	11 (30 5)	6 (24 0)	17 (27 9)
Two	10 (27 8)	3 (12 0)	13 (21 3)
Three	5 (13 9)	5 (20 0)	10 (16 4)
More than three	6 (16 7)	5 (20 0)	11 (18 0)

transanthro-sphenoidal surgery In another patient, a small mammary carcinoma was detected at the time when she was admitted for pituitary surgery

Ten of the married patients were childless, but 34% had from 3 to 9 children

The weight height, blood pressure and heart volumes are listed in Table II

Increased pigmentation was seen in 14 9% of the males and in 12 1% of the females Hypertrichosis was seen in 36 2% and in 39 4%, respectively

Increased perspiration was the most common symptom followed by paraesthesiae, headache and joint pain At the last follow-up, total regression of the main symptoms was noted in somewhat 70% of the patients (Table III) Total regression or improvement of the clinical signs and symptoms of acromegalic activity was seen in 93 8% of the patients Secondary amenorrhoea or irregular menstruation occurred in 78 7% of the women At the time of operation impotence and/or decreased sexual libido were seen in 53 2% of the male patients Psychic disturbances, mainly in the form of moderate depression, occurred in 36 2% among the male patients and in 30 3% among the females

At the time of operation signs of ACTH insufficiency occurred in 18 8% of the patients, TSH in 13 8% and ADH (antidiuretic hormone) in 7 5%

Pathological intravenous glucose tolerance test was seen in 59 5% of the patients preoperatively—the postoperative regression rate of acromegalic activity seemed to be best correlated to a restitution of normal glucose tolerance

DISCUSSION

The most commonly postulated theory of tumour genesis in the pituitary gland is that of increased hypophysiotropic stimulation from the hypothalamus—in some cases possibly induced by a primary end organ deficiency (Russfield, 1968, Pelletier, 1971, Doniach, 1972) STH uniquiely can stimulate both normal and supranormal growth Many factors are known to stimulate the STH release—among these are hypoglycaemia, prolonged fasting, exercise, anaesthesia, surgery and trauma (Glick et al , 1965, Trenkle, 1970, Carey et al , 1971, Buckler, 1972) Theoretically, head trauma may disturb the hypothalamic centre for the control of STH secretion Encephalitis and meningitis may act in the same way

In acromegaly, the frequent co-existence of goitre, the frequent occurrence of latent or manifest diabetes mellitus amenorrhoea, hyperpigmentation and asthenia suggest a participation of the thyroid, pancreatic islets, ovary and adrenal glands These symptoms have been grouped together under the heading of polyglandular syndrome (Cushing, 1912) It has been suggested that STH may produce tumours in a number of organ sys-

Table II *Height, weight, STH, blood pressure and relative heart volume in 80 acromegalic patients*

	At operation	At last follow up
Males		
Height cm	n=47 mean 179 5 range 166–207	
Weight	n=47 mean 92 2 range 66–127	n=45 mean 9? 8 range 66–137
STH ng/ml	n=38 mean 55 0 range 6–283	n=46 mean 9 7 range 1 3–50 0
Blood press *mmHg*		
systolic	n=47 mean 155 range 110–210	n=45 mean 149 range 110–200
diastolic	n=47 mean 95 range 60–160	n=45 mean 92 range 60–130
Heart vol	n=32 mean 488 range 350–720	n=18 mean 490 range 290–800
Females		
Height cm	n=33 mean 165 4 range 152–174	
Weight kg	n=33 mean 67 7 range 54–78	n=33 mean 70 3 range 54–81
STH ng/ml	n=27 mean 64 3 range 6–520	n=32 mean 50 2 range 1 6–1 000
Blood press mmHg		
systolic	n=33 mean 152 range 100–220	n=33 mean 150 range 110–200
diastolic	n=33 mean 95 range 60–125	n=33 mean 92 range 70–120
Heart vol ml/m²	n=21 mean 432 range 300–620	n=8 mean 443 range 370–530

tems, and acromegaly may be associated with pheochromocytoma, goitre diabetes mellitus and endometriosis (Miller & Wynn 1971)

Peptic ulcers antedate the pituitary symptoms by 5 to 10 years and is often fulminating and intractable (Roth & Vilardell 1960) Hyperplasia of the submandibular glands in acromegalic patients has been observed occasionally (Benda, 1901, Ardran & Kemp, 1972) —the combination of pleomorphic adenoma of the parotid gland with acromegaly has been reported by Gadrat et al , 1970

In a polysymptomatic disease as acromegaly the question must, however, always be put whether one or several of the symptoms and signs may be caused by a co existent pathological condition

From the present relatively large series no definite conclusions can be drawn as regards the influence of genetic factors in acromegaly *Such factors may, however, come to play a* greater role in the future since the fecundity among the patients seems to be good and successful pregnancies without re activation of the disease have been observed after the transanthro sphenoidal operation of the pituitary adenoma In this series the operation resulted in a high rate of regression of the acromegalic symptoms and at the same time normal anterior pituitary function was often restored

ZUSAMMENFASSUNG

Der Verfasser hat eine klinische und Nachuntersuchung an 80 Akromegalien unternommen die mit der trans antro-sphenoidalen Operation des Hypophysentumors behandelt waren Hereditat fur Akromegalie oder Gigantismus wurde in 3,8% der Patienten gefunden und an

Table III *Symptoms and signs in 80 acromegalic patients*

	At operation	At last follow up	Regression (%)
Sweating	72 (90 0)	19 (23 8)	73 6
Paraesthesia	54 (67 5)	14 (17 5)	74 1
Headache	48 (60 0)	11 (13 8)	77 1
Joint pain	37 (46 3)	10 (12 5)	72 9
Soft tissue swelling			
Face	79 (98 8)	48 (60 0)	39 3
Hands and feet	79 (98 8)	55 (68 7)	30 4
Skeletal changes			
Head and face	76 (95 0)	76 (95 0)	0
Hands and feet	71 (88 8)	71 (88 8)	0

dere hereditäre Faktoren in 13.8%, Schädeltrauma, Meningitis oder Encephalitis in 18.8%. Die dominierenden Symptome waren Schwitzen, Parästhesien, Kopf- und Gelenkschmerzen. Die Akromegalie war in 37.6% von Kropf, Parathyreoideadenome, Ulcus ventriculi oder duodeni, Parotistumor oder Schwellung der Glandula submandibularis begleitet. Ungefähr 34% der verheirateten Patienten hatten drei oder mehr Kinder. Einige Patienten hatten nach der Hypophysenoperation eine normale Schwangerschaft.

Reference list can be required from the author.

Acta Otolaryngol 82 234–237, 1976

TWO FAMILIES WITH ALPORT'S SYNDROME

L M Odkvist, P Kylen and M Lundberg

From the ENT Department Regional and University Hospital Linkoping Sweden

Abstract Alport's syndrome is a hereditary disease striking cochlea eye and kidney The diseased women usually have a nonlethal degree of the kidney disease but the prognosis for the men is worse They often die from renal failure before the age of 35 Most cases of hearing loss occurs in men The hearing loss is progressive after the age of 10 *Audiological tests are characteristic for a* cochlear lesion The debut of the disease usually appears in the post natal period giving microscopic haematuria It can however occur later with haematuria and proteinuria The hearing loss may be the first symptom which is an observandum to otologists Two families with Alport s syndrome are described The patients have been examined concerning kidney disease audiological and vestibular pathology The hereditary pattern is described

A C Alport 1927 described a family with a hereditary kidney disease and hearing loss, a syndrome that after a suggestion by Williamson (1961) carries the name Alport's syndrome This syndrome has often been studied in journals for internal medicine but heretofore the otological side of it with a few exceptions has been neglected Hence we feel it important to describe two families with Alport's syndrome to otologists

CASE REPORT

At the Renal clinic and the Audiological clinic, University Hospital in Linkoping two families with Alport's syndrome have been examined In family A (Fig 1) the primary case is a woman, now 60 years of age As far as it is known this is the first patient with signs of renal disease in the family Her symptoms are intermittent haematuria and albuminuria without *any sign of renal failure* Her hearing ability is normal She and her healthy husband have 3 children, in Fig 1 named A II, A III and A IV, who since child hood have had haematuria and proteinuria The son A III at the age of 20 developed a renal failure and succumbed after a renal transplant at the age of 26 His hearing was normal

The son, A II, at the age of 34 has a moderate renal function weakness Since the age of 10 he has had a progressive hearing loss His hearing has been checked several times since the age of 26 His hearing loss by then was a 70 dB flat loss on the right side and on the left side 40 dB His hearing loss has been progressive Speech audiometry, Bekésy audiometry and SISI give a cochlear picture His electronystagmogram is normal with symmetrical caloric reactions

A II has two daughters with normal hearing but intermittent haematuria (A V and A VI)

A daughter of the patient A I (A IV) has a *slight kidney disease without renal failure* and a normal hearing, and her daughter (A VII) has the same symptoms

According to verbal legend it is known that within the family B several relatives in former generations have died from renal failure, and hearing losses have been common

B I died 1941 from a renal disease and had a hearing loss No hearing tests were performed B I had 6 children, two with her first husband

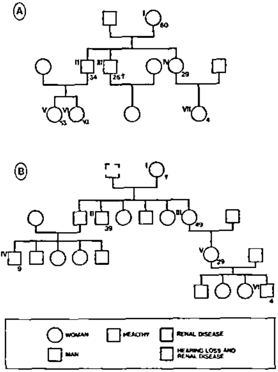


Fig 1 Pedigree for two families with Alport s syndrome

and four with her second one Both men were healthy The son B II at the age of 39 has a renal affection with slightly increased serum creatinine He has had a hearing loss since the age of 10 The hearing loss has not been progressive beyond the 60 dB level (see Fig 2) It is of a sensorineural type, audiologically giving the pattern of a cochlear lesion His nystagmogram is normal It is interesting to note that a healthy brother, B II, has a son aged 6 with renal disease His hearing is normal (B IV)

B III half sister to B II with different fathers, has a microscopic haematuria as has her daughter and grand daughter (B IV, B VI), all of them showing normal audiograms

DISCUSSION

Heredity

Several authors after having studied different families with Alport s syndrome have proposed different theories concerning how the disease is inherited (Perkoff, 1967, Stephens et al , 1951, Graham, 1959, Shaw et al , 1961, Arnott et al , 1966, Sarre et al , 1966, Karlberg, 1968) The disease is most commonly transmitted through mothers having the syndrome, but heritance through fathers may occur, and sometimes the transmittance goes through symptom-free parents No certain proof has been given for a connection between chromosome changes and Alport's syndrome, although it is likely that there are chromosome changes The gene seems to be sex-influenced and not totally penetrant

Pathogenesis

Several theories concerning the pathogenesis have been suggested by different authors Goldbloom et al (1957) suspect an inherited enzyme defect with progressive accumulation of a toxic substance Johnson & Hagan (1965) support this theory Perkoff et al (1958) are suggesting a defect lipid metabolism with secondary changes in cochlea and kidney Iversen (1974) has a theory concerning a specific collagen defect in the patients and that this defect collagen is specific for the basilar membrane in the glomerulus, cochlea and lens Hinglais et al (1972) support this

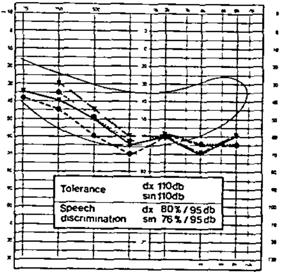


Fig 2 Audiometry findings in the patient named B II

theory concerning degenerations in collagen tissue

Morphology

Kidney The histopathological picture in the kidney usually has features in common with glomerulonephritis, interstitial nephritis and secondary pyelonephritis The glomerulonephritelike alterations consist of thickening and hyalinisation of capillary basement membrane in glomeruli Some glomeruli remain intact The characteristic sign seems to be the presence of foam cells At immuno-fluorescence microscopy no positive reactions for immuno globuline have been found Renal biopsies in our material so far has not succeeded to show any pathology

Cochlea Lachheim et al (1968) have found collagen changes in the organ of Corti in some cases Crawfurd et al (1968) in one case describe atrophy of the organ of Corti and foam cells in the saccus endolymphaticus, changes also described by Gregg & Becker (1963)

Eye Pathology in the eye is unusual and is described only in few cases The changes appear in the lens and consist of cataracts, lenticular opacities and occasionally keratoconus Some cases with retinopathy have been described (Ohlson, 1963)

Symptoms

The disease usually starts with a microscopical haematuria In the majority of the cases this haematuria exists already in the post natal period In women the disease usually includes only an intermittent microscopic haematuria and a slight proteinuria without renal failure This type of cases is common in our families A and B

Men more commonly run into a renal failure causing death before the age of 35 This is shown by case A III The hearing loss is most common in men and starts after the age of 10 as in our cases A II and B II It is important to know that the disease may start with a hearing loss without symptoms of renal disease This is pointed out by Cassady et al (1965) In our families A and B no such case is present According to the literature a debut with hearing loss is more common in some families with the disease These cases starting with the hearing loss to the otologist is an observandum not to forget questions about heredity for hearing loss and renal disease in his case history Urine tests may give the diagnosis when a case of inexplicable sensorineural hearing loss is investigated

The audiological picture is cochlear in our cases as well as in the literature The hearing loss in men usually progresses to a level that give social problems Surditas is uncommon In our families we found no lenticular pathology The first symptom noticeable when the eye gets affected usually is myopia

Therapy

At present no treatment can cure the kidney disease or prevent its progress Ordinary diet salt and water restrictions are used in cases of renal failure In several cases hemodialysis has been used Several successful renal transplants have been described in the literature, and it is also shown that patients with hereditary nephropathy has a somewhat better 2 year survival than other patients with kidney transplants (The 9th report of the Renal Transplant Registry, 1972)

Conclusion

Alport s syndrome is a hereditary disease concerning the kidney, cochlea and sometimes the eye It is important to trace the cases as the prognosis may be pessima Early diagnosis is of value, as treatment may save the patient for many years and hemodialysis and renal transplant may be necessary Knowledge concerning this hereditary disease is important in family planning Early diagnosis also gives the patient with a hearing loss the chance to get a hearing aid before he has suffered social problems due to his hearing loss

ZUSAMMENFASSUNG

Alports Syndrom ist eine erbliche Krankhe t d e an der Gehörschnecke am Auge und an der Niere auftri t Die

erkrankten Frauen haben im allgemeinen einen nicht tötlichen Grad der Nierenkrankheit wahrend die Prognose fur Manner schlechter ist Diese sterben oft an Nierenversagen bevor sie 35 Jahre alt werden Die meisten Fälle von Gehorverlust treten bei Mannern auf Der Gehorverlust ist fortschreitend nach einem Alter von 10 Jahren Audiologische Tests zeigen eine charakteristische Schneckengang Beschadigung Erstmalig tritt die Krankheit gewohnlich in der Zeit nach der Geburt als mikroskopische Hamaturia auf Sie kann spater auch als Hamaturia mit Proteinuria vorkommen Der Gehorverlust kann das erste Symptom sein welches ein Observandum for Otologen ist Es werden zwei Familien mit Alports Syndrom beschrieben Die Patienten sind wegen Nierenerkrankung audiologischer und vestibularer Pathologie untersucht worden Es wird berichtet daß die Krankheit erblich ist

REFERENCES

Alport A C 1927 Hereditary familial congenital haemorrhagic nephritis *Br M J I* 504

Arnott E J Crawfurd M D A & Toghill P J 1966 Anterior lenticonus and Alport s syndrome *Br J Ophthalmol 50* 390

Cassady G Brown K Cohen M & DeMaria W 1965 Hereditary renal dysfunction and deafness *Pediatrics 35* 967

Crawfurd M D A & Toghill P J 1968 Alport s syndrome of hereditary nephritis and deafness *Q J Med 37* 563

Goldbloom R B Fraser F C Waugh D Aronovitch M & Wiglesworth F W 1957 Hereditary renal disease associated with nerve deafness and ocular lesions *Pediatrics 20* 241

Graham J B 1959 Hereditary chronic kidney disease An alternative to partial sex linkage in the Utah Kindred *Am J Hum Genet 11* 333

Gregg J B & Becker S F 1963 Concomitant progressive deafness chronic nephritis and ocular lens disease *Arch Ophthalmol 69* 293

Hinglais N Grunfeld J P & Bois E 1972 Characteristic ultrastructural lesion of the glomerular basement membrane in progressive hereditary nephritis (Alport s syndrome) *Lab Invest 27* 473

Iversen U M 1974 Hereditary nephropathy with hearing loss "Alport s syndrome *Acta Paediat Scand* Suppl *245*

Johnson W J & Hagan P J 1965 Hereditary nephropathy and loss of hearing *Ann Otolaryngol Chir Cervicofac 82* 166

Karlberg B 1969 Alport s Syndrome *Nordisk Medicin 8?* 1254

Lachhein L Kemnitz P Buttner H Thal W & Witkowski R 1968 Erbliche Nephritis mit Innenohrschwerhorigkeit (Alport Syndrom) *Dtsch Med Wochenschr 93* 1891

Ohlsson L 1963 Congenital renal disease deafness and myopia in one family *Acta Med Scand 174* 77

Perkoff G T 1967 The hereditary renal diseases *New Engl J Med 277* 79

Perkoff G T Nugent C A Dolowitz D A Stephens F E Carnes W H & Tyler F H 1958 A follow up study of hereditary chronic nephrit *Arch Intern Med 102* 733

Sarre H Rother K Schmitt Ch Unger H H Beickert P & Baitsch H 1966 Neue Befunde zur hereditaren chronischen Nephritis (Alport Syndrom) *Arch Kl n Med 212* 1

Shaw R F & Glover R A 1961 Abnormal segregation in hereditary renal disease with deafness *Am J Hum Genet 13* 89

Stephens F E Perkoff G T Dolowitz D A & Tyler F H 1951 Partially sex linked dominant inheritance of interstitial pyelonephritis *Am J Hum Genet 3* 303

Williamson D A J 1961 Alport s syndrome of hereditary nephritis with deafness *Lancet II* 1321

The Ninth Report of the Human Renal Transplant Registry 1972 *JAMA 220* 253

Acta Otolaryngol 82 238–241, 1976

LABYRINTHINE MALFORMATIONS

Genetical and Radiological Aspects

G Everberg and J Jensen

From the ENT Department Sundby Hospital and the Radiological Department Rigshospitalet Copenhagen Denmark

Abstract A family is described three members of which presented progressive deafness of mixed type not present at birth The two living mother and daughter, ex[illegible]

The findings being unique are discussed in the light of Brown Farrior og Endicott s paper on progressive, non congenital deafness attributed to abnormally wide canaliculus cochlea

Deafness associated with macroscopically visible anomalies of the labyrinth is a particularly challenging object for research since the patho anatomical background can be studied radiologically

The Mondini malformation of the cochlea is of special interest, partly because it has so far been regarded as a well defined entity and partly because it appears quite frequently in patients with congenital deafness

The malformation is characterized by a reduction in the number of coils from 2¾ to 1½, above which a cavity is present

Definite radiological demonstration of this malformation was made by Jensen (1969), who found 5 cases among 62 deaf children No hereditary factor could be demonstrated in any of the cases

The present paper is a combined clinical, hereditary and radiological study of a family in which hearing impairment, deafness and Mondini-like malformation of the labyrinth were present (Fig 1)

The proband (AVH, 3 4 65, No 4 in the pedigree) is a 10 year old girl, the first of two children Pregnancy and delivery were uneventful, and in spite of her mother's deafness, there was no suspicion of hearing impairment during her first years of life Her speech seemed to develop late, but that was ascribed to lack of stimulus in a home in which the mother was deaf To accelerate her speech she was sent to a kindergarten where medical routine examination revealed a hearing impairment

Physical examination revealed a healthy-looking girl of normal intelligence who carried out an almost normal conversation not least due to a well developed capability of lip-reading *Otoscopy* was normal *Audiometry* (Fig 2) showed a severe hearing impairment on the left side, which in the bass seemed almost exclusively conductive, while in the treble it seemed perceptive

In the right ear a severe conductive hearing impairment was found in the bass—almost identical with the finding in the left ear In the treble, a moderate, mainly perceptive hearing impairment was found

In the *caloric-vestibular examination* irriga

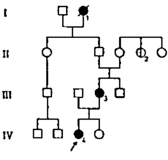


Fig 1 Pedigree of the family Nos 1 3 and 4 affected members No 2 right sided deafness from temporal fracture (hospital case record)

tion of the right ear with 30°C water released a rapid horizontal nystagmus to the left of 130 seconds' duration On the left side, there was no reaction after irrigation with 10°, 30° and 44° water

Radiological examination Tomography was performed with the Philips Massiot Polytome (hypocycloid blurring) For the evaluation of the outer ear, middle ear, the semicircular canals and the internal acoustic meatus the antero posterior projection was used and the structures mentioned were all normal apart from a slight widening of the lateral semicircular canals

For the evaluation of the cochlea the axial-pyramidal projection was employed On the right side, a normal basal and a normal middle coil were found The apical coil could not be seen The canaliculus cochleae was of normal width On the left side (Fig 3), a normal basal coil was found Above this, a cavity, representing the two upper coils, was seen The canaliculus cochlea was wider than on the right side, and wider than normal

The *proband's mother* (MH, 19 9 36) is a 38-year-old woman, who in her first years of life developed normally Until the age of three she heard the door bell and the telephone and she learnt the usual words and small sentences, but her speech development seemed delayed, and she did not learn any songs (melodies) Consequently, she was sent to a kindergarten, where her hearing impairment was detected The hearing grew worse, and she spent her school time in a school for deaf children

Physical examination revealed normal otoscopic conditions *Audiometry* showed an almost total deafness with a threshold of 80–100 dB

Caloric vestibular examination showed no reaction on either side to irritation with 10°, 30° and 44° water.

Radiological examination On the right side, the anteroposterior projection revealed a normal tympanic cavity, normal semicircular canals and a normal internal acoustic meatus The ossicles proved to be normal in the lateral projection The axial-pyramidal projection showed a two-storey cochlea, but no cavity The canaliculus cochleae could not be identified

On the left side, the anteroposterior projection (Fig 4) showed a normal external acoustic meatus The tympanic cavity was reduced in height—no attic was present The vestibular and semicircular canals were normal, but the level of the lateral semicircular canal was abnormally high, above the floor of the middle cranial cavity The ossicles were malformed and appeared as irregular osseous structures

The axial-pyramidal projection showed a normal basal coil above which a cavity was found, just as in the proband's left ear The de

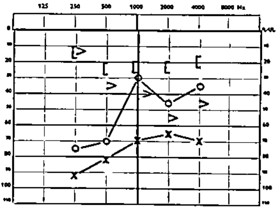


Fig 2 Audiogram of the proband



Fig 3 Ax al pyram dal view of the proband s left ear show ng the Mond ni cav ty in the cochlea

ceased paternal grandmother of this woman was allegedly hard of hearing from her youth although she had worked as a saleswoman From the age of 50 she had been nearly deaf and had to wear a hearing aid (No 1 in the pedigree)

DISCUSSION

This family has three deaf members and the two living mother and daughter present apparently genetically caused malformations of the labyrinth

Audiologically and radiologically the two patients are peculiar It is noteworthy that the hearing impairment was discovered at the age of three in the mother and at the age of five in the proband Still more unusual is it that the threshold in the proband was found fluctuating with a tendency of progression during the five year period in which annual audiometry has been performed This phenomenon was at first ascribed to uncertainty in the child However similar progression of the hearing impairment was as already mentioned noticed in the other two family members

Brown Farrior & Endicott (1971) gave a detailed description of some cases with congenital progressive mixed hearing impairment which were not noticed until the age of 2–3 years About 20 cases from the literature were reviewed and the authors added another six

Tomography of our patients did not reveal any labyrinthine dilation but the canaliculus cochlea was dilated in the proband s left ear

The deafness observed may possibly be of the type described by Brown Farrior & Endicott (1971) apart from the absence of labyrinthine dilatation The simultaneous occurrence of a unilateral Mondini malformation (which in itself is unusual) and middle ear malformation in the mother render the findings peculiar —and prohibits classification into any system so far known

In Guffarth s thesis (1936) six cases of the Mondini malformation from the literature were classified genetically as heredolabyrinthine degeneration with irregular dominant heredity A similar heredity was found in the family considered in the study reported here



Fig 4 Antero-posterior view of the mother s left ear Note the low lying tegmen and the irregular osseous structures in the tympan c cav ty represent ng the malf rmed oss cles The vest bule and semic rcular canals are normal

ZUSAMMENFASSUNG

Es wird von einer Familie berichtet, in der es drei Mitglieder gab, die nicht angeborene, vorwartsschreitende Taubheit gemischten Typus darboten Die zwei leben den, Mutter und Tochter, boten eine Mondini ähnliche Mißbildung des Labyrinthes dar Canaliculus cochleae war bei der Tochter erweitert Außerdem gab es bei der Mutter eine Mittelohr Mißbildung Diese einmaligen Beobachtungen sind auf Grund der Abhandlung von Brown Farrior und Endicott besprochen in der solche vorwartsschreitende, nichtangeborene Taubheit einem erweiterten Canaliculus cochleae zugeschrieben wird

REFERENCES

Brown Farrior, I & Endicott, J 1971 Congenital mixed deafness, cerebrospinal fluid otorrhoea Ablation of the aqueduct of the cochlea *Laryngoscope 81*, 684

Jensen, J 1969 Malformations of the inner ear in deaf children *Acta Radiol* Suppl *286*

Guffarth, F 1936 Das path -anat Bild der hereditär-degenerativen Innenohrschwerhorigkeit bzw Taubheit Tubingen

Acta Otolaryngol 82 242–244 1976

CONGENITAL PERCEPTIVE HEARING LOSS AND ATOPIC DERMATITIS

G Frentz G Everberg and H C Wulf

From the Department of Dermatology the Finsen Institute and the Otolaryngological Department Sundby Hospital Copenhagen Denmark

Abstract Two brothers were suffering from perceptive hearing loss atopic dermatitis and mild palmoplantar keratoderma. There was a predisposition to atopic disease in the maternal family and palmoplantar keratosis as a dominant trait in the paternal family Atopic dermatitis and palmoplantar keratoderma separately have been reported to co-exist with hearing loss but a combination of all three has not so far been reported

In 1968 Konigsmark et al published a survey of hereditary syndromes with auditory and dermatological manifestations and at the same time presented a report of a family in which 3 out of 4 siblings exhibited perceptive hearing loss and atopic dermatitis Apparently no such cases have been published since then We feel it must be of interest to report a similar syndrome in two brothers in whom it co-existed with palmoplantar keratosis a combination not previously described

Case history

The propositus was a boy aged 9 years the younger of a family of two admitted to a dermatology unit with atopic dermatitis From the age of 3 months he had been suffering from itching eczema on the cheeks spreading to the arms and legs involving especially the flexor surfaces He had always had dry skin with highly increased volar and plantar keratosis After severe bilateral perceptive hearing loss had been observed at the age of 4 he had been wearing hearing aids on both sides No abnormalities of the teeth nails or perspiration were observed

Rhinoscopy showed varying appearances mucous secretion being present from time to time In spring the mucosa was pale cyanotic and covered with clear secretion as in allergic rhinitis *Otoscopy* and *caloric vestibular* tests were normal *Audiometry* revealed pronounced bilateral symmetrical perceptive hearing loss mainly at the middle frequencies (Fig 1) thus appearing as a basin curve typical of certain types of hereditary perceptive hearing loss

Dermatological examination showed that the skin was on the whole dry with minor papules thickened with coarse grooves and lichenization around the mouth on the instep the dorsal aspect of the hands and feet including fingers and toes typical of atopic dermatitis

On the abdomen there was mild ichthyosis with delicate scaling the palms and soles had accentuated lines Several fissures in the region of the heel and distinct hyperkeratinization of the palms and soles were present The hair line was abnormally low at the back and temples and the extremities showed moderate hypertrichosis The skull external ears eyes and teeth were of normal shape

Other investigations IgE 1580 arbitrary units (normal range 30–500) Examination for allergy using scratch tests showed a positive

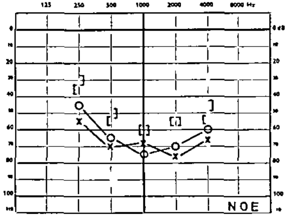


Fig 1 Audiogram of the propositus

reaction to several grass pollens ox eye daisy and house dust mite, doubtfully positive reactions to rush and barley

Chromatographic study of the amino acid content in the urine showed normal levels Chromosome study normal complement 46 XY (preparation by machine, counting of 25 cells, one trypsin-Leismann stained karyotype was made)

Family history and findings

The patient's brother aged 13 years, had a similar skin affection, but less severe The history and appearances were suggestive of atopic dermatitis and mild keratoderma

No signs of hay fever or asthma Scratch tests proved positive to house dust several grass and tree pollens and animal hair

At the age of 6 years adenoidectomy had been performed At the same time, audiometry had revealed moderate bilateral symmetrical perceptive hearing loss presenting the same basin curve as seen in his younger brother Subsequent audiograms showed no progression of hearing loss

Otoscopy rhinoscopy and vestibular tests showed normal conditions Chromosome complement 46 XY

No other members of the family had hearing loss The mother a maternal aunt and the maternal grandmother had a low hair line The mother had been afflicted with severe atopic dermatitis and still suffered from hay fever She exhibited changes indicative of volar and plantar ichthyosis, but no keratoderma Scratch tests were highly positive to several grass pollens and to cat The maternal grand father also had a history of hay fever, and in his youth he had been admitted to hospital with eczema, morphologically consistent with late atopic dermatitis

Several members of the paternal family had dry skin and abnormally pronounced volar and plantar keratinization (see pedigree, Fig 2) None of them had consulted a specialist, but the father had changed his occupation because of the skin condition Examination of the father showed accentuation of the volar and plantar lines and marked, widespread hyper keratinization in the same areas, with a few solitary punctiform hyperkeratotic lesions

The parents were not related

DISCUSSION

The two boys, of a family of two, were suffering from bilateral perceptive hearing loss and atopic dermatitis The same syndrome was found by Konigsmark et al in 3 out of 4

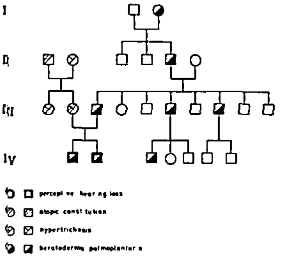


Fig 2 Pedigree of the family

siblings As in our family, no further case of hearing loss could be found in the family, and the disease was interpreted as a recessive trait Unlike our cases the skin disease was atypical in localization and time of onset, and there seemed to be no further familial cases of atopic or other ectodermal anomalies

In our family, the skin disease manifested itself as typical atopic dermatitis with positive scratch tests The atopic predisposition was inherited from the maternal family The mode of inheritance of the atopic predisposition has not been clearly established, but several authors claim to have found evidence of multi factorial inheritance

The paternal branch of the family had palmoplantar keratoderma of dominant in heritance, also present in a mild degree in both the children The dominantly inherited form of palmoplantar keratoderma is allegedly rare ly associated with other ectodermal anoma lies, but the literature contains examples of cases combined with onychodystrophy, leuco nychia, hyperhidrosis, and perceptive hearing loss It is beyond doubt that the two brothers were suffering from congenital familial per ceptive hearing loss At the same time they had atopic dermatitis and a mild degree of palmoplantar keratoderma The co-existence of these disorders might be due to mere chance but judging from the literature it seems reasonable to believe that the disorders were genetically associated This syndrome has not been described previously

ZUSAMMENFASSUNG

Zwei Bruder litten an Innenohrschwerhörigkeit atopischer Dermatitis und leichter Keratosis palmoplantaris Mutterlicherseits war eine Anlage fur atopische Leiden vorhanden und vaterlicherseits kam Keratosis palmoplantaris in dominant erblicher Form vor Sowohl atopische Dermatitis als auch Keratosis palmoplantaris sind in der Literatur mit Innenohrschwerhorigkeit zusammen beschrieben worden wahrend eine Kombination der drei erwahnten Leiden vorher noch nicht berichtet worden ist

REFERENCE

Konigsmark B W Hollander M B & Berlin C I 1968 Familial neural hearing loss and atopic dermatitis *JAMA 204* 953

Acta Otolaryngol 82 245–248, 1976

IDENTIFICATION OF NORMAL HEARING CARRIERS OF GENES FOR DEAFNESS

H Anderson and E Wedenberg

From the Department of Audiology Karolinska Sjukhuset Stockholm Sweden

Abstract The dominant and sex linked forms of hereditary hearing loss which have long been recognized are readily identified on the basis of the family history and routine hearing tests The mode of inheritance of the recessive forms of hereditary deafness on the other hand has been extremely difficult to determine The research of the last few years however has disclosed that carriers of genes for recessive deafness can be identified by audiometric recording of certain peculiarities in the hearing function This is an important advance not only as regards diagnostic work but also in the research into the genetics of deafness

In 1908 Sir Archibald Garrod the English physician, coined the term inborn errors of metabolism for a pathological entity comprising the four diseases albinism alkaptonuria cystinuria and pentosuria They had certain features in common

1 They could be demonstrated in the first days of life
2 They tended to occur frequently in certain families in a large number of cases
3 They were fairly benign and compatible with a normal length of life
4 They often appeared in the offspring of consanguineous marriages

Garrod s prophecy that the underlying cause of these diseases would prove to be an enzymatic defect has proved correct From the original number of four the inborn errors have by now increased to more than a hundred Hereditary deafness satisfies the four postulated conditions for inborn errors of metabolism

Provided that the tests are subtle enough many hereditary diseases may be disclosed in their symptom free carriers by minor deviations from normal If a person is a heterozygote for a complaint, he has one normal and one mutant gene Since each gene is responsible for the formation of its own protein, which is often an enzyme, in the case of a changed genetic structure the mutant gene will either have prevented the formation of its own protein or it will have given rise to an abnormal enzyme

There are, however, no biochemical tests by which it is possible to detect any abnormal activity in enzymes responsible for the development and function of the auditory system For this reason it has not been possible to class hereditary deafness as an inborn error of metabolism, but this does not rule out the possibility that it belongs to this group it means only that our knowledge of the involvement of these enzymes in the biological process is still too limited for us to be able to ascertain the biochemical basis of these defects

There remains the possibility of demonstrating the effect of any enzymatic defects on the hearing organ in gene carriers by audiometric studies Such attempts have been made in order to detect heterozygotes among the parents of children considered to have a genetic hearing impairment (Wildervanck, 1957) From a study of 15 normal hearing parental

pairs with their 32 children with recessive deafness, Wildervanck inferred that it was impossible by ordinary audiometry to identify these parents as heterozygotes "The conclusion of my entire investigation is that normal hearing parents of children with deafness are not detectable as heterozygotes" Nor could Fraser find any significant hearing impairment in heterozygotes in sex-linked recessive deafness It may thus be assumed that any hearing defects in the heterozygotes are subclinical, and that to obtain a solution of the problem more refined methods than have been used so far must be applied

It has always been extremely difficult in the individual case to distinguish between hearing impairments due to endogenous and exogenous factors, respectively

The differential diagnosis has usually been carried out on the basis of routine hearing tests and medical histories, especially the family history "Look at the family rather than at the individual", wrote Alexander Graham Bell in 1891, but there is then the difficulty of weighting anamnestic information on deaf relatives against the objective results of examination and the possible effect of any exogenous factors that have a bearing on the hearing before during or after birth

In short the consensus is that the hereditary group comprises about one half of all children with severe hearing impairment In the hereditary group the number of cases with a dominant mode of inheritance is very small Only about 5 per cent of the children in schools for the deaf are born in marriages where one or both parents are deaf Carriers of dominant genes for deafness are readily identified by audiometry

However, by far the largest group is that in which normal hearing parents give birth to one or more children with hearing impairments as the sole symptom, and in which the hearing defect is judged to be of the hereditary recessive type Usually, these children show a fairly rapid progress of the hearing loss most pronounced for the higher frequencies The de-

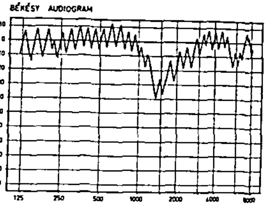


Fig 1 Example of dip in hearing threshold recorded by Békésy audiometry Note that its position is such that the significant characteristic of the dip might be missed in routine octave audiometry

fect would appear to be due to an autosomal recessive gene, the deaf children would then be homozygotes and the parents heterozygotes However, because of the difficulty of accounting for the paradox that parents suffering from recessive deafness usually produce normal hearing children, it has been proposed that recessive hearing impairment may be caused by several genes located in different loci The suggested number of such loci varies widely and have been estimated to be not less than 36 (Chung et al, 1959) and "at least 45" (Sank, 1963) These views are, however, based on studies performed only by octave audiometry and prior to the introduction of more modern methods One of the great problems has been to find a method that is sensitive enough to disclose even the gene carriers

To obtain a better understanding of the genetics in recessive deafness a family study of 30 parental pairs was carried out at the Karolinska Hospital The parents, who all demonstrated clinically normal hearing had 74 children, 47 of whom had a severe hearing loss with an established hereditary aetiology The object of the study was to investigate the possibility of identifying the suspected gene carriers by more advanced methods of hearing tests One such technique is Békésy audio-

metry, in which the continuous recording enables even minor peculiarities or deviations in the hearing threshold to be detected The other method used was recording of the stapedius reflex threshold As the stapedius reflex is elicited at rather high sound intensities, the ear by this test is submitted to a certain strain It was presumed that this supra-threshold test—"working audiometry"—might reveal defects in the hearing function that could not be detected by the hearing threshold tests Moreover, the stapedius reflex test has the advantage of being completely objective

The Bekesy audiometry showed that in 30% of the marriages at least one parent revealed a "dip" in the middle frequency range of the hearing threshold (at about 1 5 kHz) of at least 20 dB and covering a range of at least one octave Such threshold dips are extremely rare in normal series, less than 2% Moreover, the stapedius reflex threshold was pathologically high in at least one member in practically all the parental pairs In the most pronounced cases, the reflex threshold was too high to be reached at some of the test frequencies a remarkable condition in view of the completely normal hearing threshold In normal series, pathologically high reflex

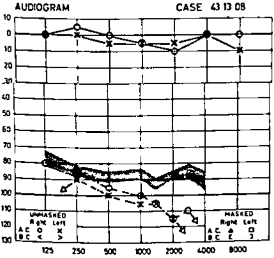


Fig 2 Example of abnormally high reflex thresholds (– –) Shaded area indicated normal range

thresholds are encountered in only 3% of the subjects Although they had clinically normal hearing, it was possible by means of Bekesy audiometry and stapedius reflex test, to demonstrate peculiarities in the hearing function in practically all the presumed carriers of genes for deafness On the basis of these results it would seem logical to conclude that the encountered peculiarities in hearing function are characteristic of carriers of genes for recessive deafness (Andersson & Wedenberg, 1968)

It was thus possible in this series to identify audiometrically such carriers in a remarkably large proportion of the subjects The recessive types of deafness, however, constitute a heterogeneous group, and no doubt there are variants that cannot be detected by the methods described here The high percentage of carriers recognized suggests, however, that the type identified represents the commonest form of recessive deafness This may then be designated as autosomally recessive or perhaps rather as intermediary, since it was still possible to detect certain peculiarities in the hearing function of the carriers In contrast to earlier studies by statistical–genetical methods applied to large series, it would now for the first time seem possible to arrive at a hearing genetic diagnosis in the individual case This advance is, of course of great value in the clinical diagnosis and, moreover, it constitutes a most important step forward in the research in the genetics of deafness

ZUSAMMENFASSUNG

Die dominanten und geschlechtsgebundenen Formen erblicher Schwerhörigkeit die man seit langem kennt sind an Hand der Familienanamnese und der üblichen Gehörsuntersuchungen recht einfach zu identifizieren Den Modus der Vererbung rezessiver Formen erblicher Schwerhörigkeit zu bestimmen ist dagegen außerordentlich schwierig Aus den Forschungsergebnissen der letzten Jahre hat sich nunmehr ergeben daß die Träger von Genen rezessiver Schwerhörigkeit durch rein audiometrische Feststellung bestimmter Besonderheiten in der Funktion des Gehörs identifiziert werden können was

einen wesentlichen Fortschritt nicht nur hinsichtlich der Diagnostik sondern auch mit Hinblick auf die weitere Erforschung der Vererbung der Schwerhorigkeit bzw Taubheit bedeutet

REFERENCES

Anderson H & Wedenberg E 1968 Audiometric identification of normal hearing carriers of genes for deafness *Acta Otolaryngol* (Stockh) *65* 535

Bell A G 1883 Upon the formation of a deaf variety of the human race *Mem Nat Acad Sci 2* 177

Chung C S Robinson O W & Morton N E 1959 A note on deaf mutism *Ann Hum Genet 23* 357

Garrod A E 1908 *Inborn errors of metabolism Lancet II* 73 142 214

Sank D 1963 Genetic aspects of early total deafness Family and mental health problems in a deaf population New York Columbia University

Wilderwanck L S 1957 Audiometric examination of parents of children deaf from birth *Arch Otolaryngol 65* 280

Acta Otolaryngol 82 249–251, 1976

MUCOUS ELEMENTS IN THE AIRWAYS

M Tos

From the ENT University Clinic Gentofte Hospital Copenhagen Denmark

Abstract Based on studies of the mucosa in foetuses in fants children and adults using whole mount methods the mucous gland and goblet cell density in the nose rhinopharynx pharynx hypopharynx and trachea is de scribed Glandular density was highest in the nose lowest in the trachea where the glands are considerably larger than in the nose The nasal pharyngeal and hypopharyn geal glands contain more serous elements than do the tracheal ones The goblet cell density is greatest in the lateral wall and the roof of the rhinopharynx smallest in the anterior part of the nose In acute and chronic tracheal disease and on constant exposure to dust the goblet-cell density is increased

The *pathophysiology of the respiratory mu* cosa depends in particular on the production of mucus To be able to evaluate the mucus producing capacity under normal and patho logical conditions information is required on the density, size and activity of the mucous glands and goblet cells

MATERIAL AND METHODS

The whole mucosa of the organ was cut free and stained by the PAS, the PAS alcian blue or the osmium tetroxide whole-mount method The nose was studied in 56 foetuses and pre mature infants and 10 adults, the rhino-pharynx, pharynx and hypopharynx in 60 foe tuses and premature infants and 12 adults the trachea in 200 foetuses and premature infants 13 children and 80 adults

RESULTS

Nose

Glands The respiratory region is provided with numerous *small seromucous* tubulo-alveolar glands The anterior part of the septum and the lateral wall contain 100–160 *anterior nasal glands* (Bojsen-Møller, 1965) with 3–20 mm long ducts, which run backwards The olfactory region contains the glands of Bowman, they are small, tubulous and serous Glandular development starts in the nasal vestibule at the 11th week of gestation, first the anterior later the seromucous glands and, at the 15th week the glands of Bowman The glands spread backwards according to a certain pattern (Tos & Poulsen, 1975) until, at the 30th week they are dis persed all over the nose In adults, the density is 6–10 glands per square mm Glandular orifices are distributed regularly all over the respiratory region (Fig 1) The glandular mass is distributed according to the space available during the development The first glands to develop have grown deepest into the lamina propria and form the basement layer of glands Glands developing later in foetal life do not grow into the depth before division and form a subepithelial layer consisting chiefly of smaller tubulo-alveolar glands

Goblet cells The respiratory epithelium which during foetal life spreads backwards from the nasal vestibule according to a certain pattern (Poulsen & Tos 1975) contains goblet cells The density of the goblet cells is greatest at the beginning of the development, it diminishes gradually as the nose grows to reach a minimum at the 30th week, and then it increases again In the adult, the goblet cells are distributed irregularly with considerable individual variations in density Mean density, however increases gradually antero-posteriorly with 4800 cells per square mm in the

Acta Otolaryngol 82 252–255 1976

LONG TERM TREATMENT OF NASAL POLYPS WITH BECLOMETHASONE DIPROPIONATE AEROSOL

I *Treatment and Rationale*

N Mygind, S Prytz, H Sørensen and C B Pedersen

From the University ENT Clinics Rigshospitalet and Kommunehospitalet Copenhagen Denmark

Abstract Beclomethasone dipropionate is a potent corticosteroid which when applied by a pressurized metered dose aerosol in the airways has pronounced anti allergic effects with no risk of systemic steroid side effects at the recommended dosage An impressive effect in hay fever and perennial rhinitis has been demonstrated A double blind short term trial showed that the drug was effective in about 80% of patients suffering from moderate to severe nasal polyposis A study of the long term effects on the nasal mucosa is indicated

Corticosteroids were introduced in the treatment of allergic airway diseases in 1950, and since then many attempts have been made to apply the drugs directly to the mucous membranes in order to obtain the therapeutic effect without systemic side effects These attempts failed either because the treatment was ineffective or because the corticosteroid was absorbed from the mucous membrane Dexamethasone phosphate aerosol in a daily dose of 1 000 μg has been shown to be effective in the treatment of hay fever (Norman & Winkenwerder 1965) but this treatment reduces the urinary excretion of 17 ketogenic steroids indicating some systemic effect (Norman et al 1967) Although this therapy has not been shown to have any advantage over oral corticosteroids, it has been used for about 10 years, especially in the USA No serious local side effects have occurred (Norman personal communication)

Beclomethasone dipropionate (Bdp) is a synthetic corticosteroid which differs in several respects from the natural hormone This modified molecule has an anti inflammatory effect 5 000 times stronger than hydrocortisone as estimated by the compound s ability to cause cutaneous vasoconstriction (McKenzie test) (Martin et al , 1975) In 1967, it was shown to be effective in the treatment of eczemas

Despite negative results with other corticosteroids Bdp aerosol was tried in asthmatic patients The results were convincing and unequivocal a daily dose of 400 μg Bdp did not affect the plasma cortisol and had an anti asthmatic effect approximating that of 5–10 mg prednisolone orally As an irrefutable proof of the drug s local mode of action several patients developed allergic skin and nasal symptoms when changing from oral prednisolone to the aerosol (Brown et al 1972 Lal et al , 1972 Clark 1972) The unique properties of this corticosteroid molecule are probably due to a combination of its exceptionally high anti inflammatory activity and its rapid enzymatic degradation to the less active beclomethasone monopropionate and the almost inactive beclomethasone (Martin et al , 1975)

The success with inhaler Bdp naturally led to a trial of intranasal Bdp in allergic nasal disease In hay fever the effect was striking in nearly all patients both adults (Mygind

1973) and children (Prahl et al 1975) The effect on the nasal symptoms appeared to be more pronounced than that of dexamethasone phosphate, and there was no significant reduction in the excretion of 17 ketogenic steroids As there was no systemic activity eye symptoms had to be treated with eye drops This combined local treatment rendered antihistamine tablets almost superfluous Bdp treatment was effective throughout the 24 hours, thus preventing the usual early morning attack of sneezing

Bdp treatment was effective in about 75% of patients suffering from perennial rhinitis (Gibson et al , 1974, Hansen & Mygind, 1974) Even though the therapeutic effect was not so marked as in hay fever the results were more impressive in these chronically troubled patients

Dose–response studies in hay fever (Andersen et al 1975) and in perennial rhinitis (Wihl & Malm 1975) indicate that 400 μg/day is more effective than 300 μg and 200 μg Treatment should therefore start with 400 μg/day and continue with this dosage until maximum effect has been obtained After that the patients can be instructed to adjust the dosage downwards until their symptoms are just controlled

Traditionally nasal polyps belong to the group of allergic nasal diseases even though it is seldom possible to demonstrate allergy Nasal polyps are rarely found in young patient with rhinitis and extrinsic asthma with proven allergy Nasal polyps may be regarded as the nasal equivalent to intrinsic asthma with onset in middle age in non atopic patients

Although allergy is seldom demonstrable in patients with nasal polyps considerable amounts of IgE are synthesized in the polyps (Donovan et al 1970) and in the nasal mucosa (Mygind et al 1975*b*) When allergy is demonstrated the specific IgE antibodies the reagins are found in a higher concentration in the polyps than in the serum which suggests local synthesis (Berdal 1954) In the majority

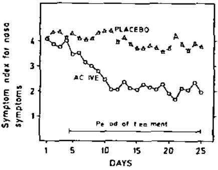


Fig 1 Average daily symptom scores (sneezing nasal secretion and blockage) during treatment with beclomethasone dipropionate and placebo in patients suffering from nasal polyps

of cases the inflammatory reaction in the polyps is allergic in type, showing local eosinophilia

A 3 week double blind study was undertaken in 35 patients with moderate to severe nasal polyps (Mygind et al 1975*a*) The effect was marked in 15 out of 19 (80%) of patients treated with the active drug (Bdp) and in 2 out of 16 (13%) in the placebo group (p<0 002) see Fig 1

The pathological changes in the nasal mucosa of patients with polyps are pronounced and only slowly reversible It follows that long term studies are necessary to ascertain the place of Bdp in treatment On the completion of the short term trial the patients were transferred to long term treatment the results of which will be presented by Pedersen et al (1976)

Having established that Bdp has an impressive beneficial effect on allergic nasal disease we decided to investigate the long term effect of the compound on the nasal mucosa When potent corticosteroids have been used in other locations (skin eyes) their use has had to be balanced against the risk of local side effects A fundamental effect of corticosteroids is inhibition of protein synthesis at cellular level There is no reason to assume that this effect and the consequent risk of local side effects are absent from the nasal mucosa However

Acta Otolaryngol 82 252-255, 1976

LONG-TERM TREATMENT OF NASAL POLYPS WITH BECLOMETHASONE DIPROPIONATE AEROSOL

I Treatment and Rationale

N Mygind S Prytz, H Sørensen and C B Pedersen

From the University ENT Clinics Rigshospitalet and Kommunehospitalet Copenhagen Denmark

Abstract Beclomethasone dipropionate is a potent corticosteroid which when applied by a pressurized metered dose aerosol in the airways has pronounced anti allergic effects with no risk of systemic steroid side effects at the recommended dosage An impressive effect in hay fever and perennial rhinitis has been demonstrated A double blind short term trial showed that the drug was effective in about 80% of patients suffering from moderate to severe nasal polyposis A study of the long term effects on the nasal mucosa is indicated

Corticosteroids were introduced in the treatment of allergic airway diseases in 1950, and since then many attempts have been made to apply the drugs directly to the mucous membranes in order to obtain the therapeutic effect without systemic side effects These attempts failed either because the treatment was ineffective or because the corticosteroid was absorbed from the mucous membrane Dexamethasone phosphate aerosol in a daily dose of 1000 μg has been shown to be effective in the treatment of hay fever (Norman & Winkenwerder, 1965) but this treatment reduces the urinary excretion of 17 ketogenic steroids, indicating some systemic effect (Norman et al 1967) Although this therapy has not been shown to have any advantage over oral corticosteroids, it has been used for about 10 years, especially in the USA No serious local side effects have occurred (Norman personal communication)

Beclomethasone dipropionate (Bdp) is a synthetic corticosteroid which differs in several respects from the natural hormone This modified molecule has an anti inflammatory effect 5000 times stronger than hydrocortisone as estimated by the compound s ability to cause cutaneous vasoconstriction (McKenzie test) (Martin et al , 1975) In 1967, it was shown to be effective in the treatment of eczemas

Despite negative results with other corticosteroids Bdp aerosol was tried in asthmatic patients The results were convincing and unequivocal a daily dose of 400 μg Bdp did not affect the plasma cortisol and had an anti asthmatic effect approximating that of 5-10 mg prednisolone orally As an irrefutable proof of the drug's local mode of action several patients developed allergic skin and nasal symptoms when changing from oral prednisolone to the aerosol (Brown et al , 1972, Lal et al 1972, Clark 1972) The unique properties of this corticosteroid molecule are probably due to a combination of its exceptionally high anti-inflammatory activity and its rapid enzymatic degradation to the less active beclomethasone monopropionate and the almost inactive beclomethasone (Martin et al , 1975)

The success with inhaler Bdp naturally led to a trial of intranasal Bdp in allergic nasal disease In hay fever, the effect was striking in nearly all patients, both adults (Mygind

Hansen I & Mygind N 1974 Local effect of intranasal beclomethasone dipropionate aerosol in perennial rhinitis *Acta Allerg 29* 281

Lal S Harris D M Bhalla K K Singhal S N & Butler A G 1972 Comparison of beclomethasone dipropionate aerosol and prednisolone in reversible airways obstruction *Br Med J III* 314

Martin L E Harrison C & Tanner R J N 1975 The metabolism of beclomethasone dipropionate in animal and man *Postgrad Med J 51* 11

Mygind N 1973 Local effect of beclomethasone dipropionate aerosol in hay fever *Br Med J IV* 464

Mygind N Pedersen C B Prytz S & Sørensen H 1975*a* Treatment of nasal polyps with intranasal beclomethasone dipropionate aerosol *Clinical Allergy 5* 159

Mygind N Weeke B & Ullman S 1975*b* Quantitative determination of immunoglobulins in nasal secretion *Int Archs Allergy Appl Immunol 49* 99

Normann P S & Winkenwerder W L 1965 Suppression of hay fever symptoms with intranasal dexamethasone aerosol *J Allergy 36* 284

Normann P S Winkenwerder W L Agbayani B F & Migeon C J 1967 Adrenal function during the use of dexamethasone aerosols in the treatment of rag weed hay fever *J Allergy 40* 57

Pedersen C B Mygind N Sørensen H & Prytz S 1975 Long term treatment of nasal polyps with beclomethasone dipropionate aerosol II Clinical results *Acta Otolaryngol* (Stockh) *82* 256

Poynter D Spurling N W 1975 Toxicity trials with beclomethasone dipropionate in the dog with particular reference to the respiratory tract *Postgrad Med J 51* 27

Sørensen H Mygind N Pedersen C B & Prytz S 1976 Long term treatment of nasal polyps with beclomethasone dipropionate aerosol III Morhpological studies and conclusions *Acta Otolaryngol* (Stockh) *82* 260

Thiringer G Eriksson N E Malmberg R Svedmyr N 1975 Bronchoscopic biopsies of bronchial mucosa before and after beclomethasone dipropionate therapy *Postgrad Med J* In press

Wihl J Å & Malm L 1976 Intranasal beclomethasone dipropionate aerosol in vasomotor rhinitis *Acta Allerg* In press

Acta Otolaryngol 82 256–259, 1976

LONG TERM TREATMENT OF NASAL POLYPS WITH BECLOMETHASONE DIPROPIONATE AEROSOL

II Clinical Results

C B Pedersen, N Mygind, H Sørensen and S Prytz

From the University ENT Clinics Kommunehospitalet and Rigshospitalet Copenhagen Denmark

Abstract The effect of a one year treatment with intra nasal beclomethasone dipropionate in 33 patients with long standing nasal polyps was determined About 80% of the patients obtained good or excellent relief of symptoms No adverse reactions of any importance occurred It is suggested that intranasal beclomethasone dipropionate is close to the ideal basic treatment for patients with nasal polyps

This study was designed to investigate the effect of beclomethasone dipropionate (Bdp) on the symptoms and the respiratory mucous membrane of patients with nasal polyps The symptomatic results are reported here, morphological and some immunological aspects of treatment will be reported in a subsequent paper (Sørensen et al 1976)

MATERIAL AND METHODS

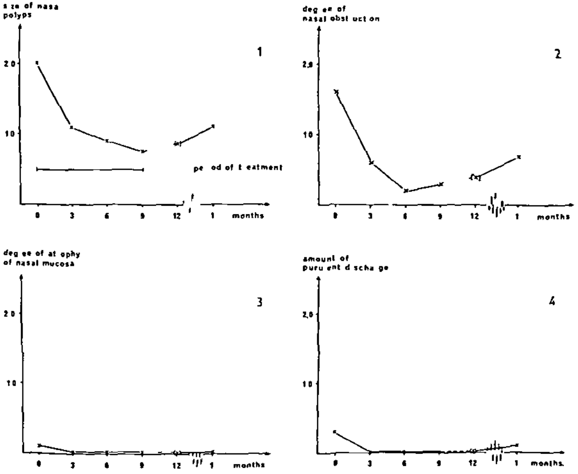
In the preceeding double blind study (Mygind et al , 1975) Bdp effectively relieved symptoms in patients treated with the active drug

Thirty three of the 35 patients from the short term trial continued in the study One patient who had received placebo did not wish to be treated with Bdp and a second successfully treated with Bdp could not participate for practical reasons The average age was 50 years (range 19–78), 22 males and 11 females participated The average duration of the disease was 15 years (range 1–60) The activity of the disease can to some extent be evaluated by the number of polypectomies On average the patients had had 8 polypectomies before starting treatment with Bdp

The atopic (allergic) state of the patients was assessed Six patients had a history of asthma eight had a family history of allergy six had a positive skin test by intracutaneous or prick testing with a series of at least 20 allergens Only one patient had a raised serum IgE the mean value being 131 U/ml, which did not differ from the normal The mean pre treatment eosinophil count was 330/μl (normal 180/μl) 26 patients had eosinophilia in their nasal smears ($\geq$10%)

The aerosol was the standard metered dose Becotide Inhaler® equipped with a special nasal adaptor It delivered 50 μg per puff, the dose was one puff into each nostril four times daily—a total dose of 400 μg/day This was the only medication given during the treatment period of 9–12 months After discontinuing treatment the patients were observed for up to 3 months

The clinical results of the treatment were estimated by the patients and by the investigator Each week the patients completed symptom cards in which the symptoms—sneezing, obstruction and rhinorrhoea—were evaluated on a scale graduated from zero to three A final statement of the overall success of the treatment was made by the patients The patients were rhinoscoped at monthly in



Figs 1-4 Results of beclomethasone dipropionate treatment estimated during rhinoscopy Two-thirds of the patients were treated for a period of 9 months and the rest for 12 months All patients were examined one month after cessation of treatment

tervals The following factors were assessed polyp size degree of obstruction secretion appearance and signs of atrophy (dry mucous membrane with crust formation in the posterior part of the nasal cavity) and scored on a scale from zero to three Nasal secretions were cultured for bacteria and fungi just before the end of treatment The results were compared with those obtained in untreated control patients

RESULTS

In 26 patients the symptoms were satisfactorily controlled by Bdp alone four patients needed supplementary treatment and in three the drug was ineffective A reduction in the size of the nasal polyps occurred during treatment The rhinoscopy findings are shown in Fig 1 There was a marked reduction in the degree of nasal obstruction which continued for several months of the treatment period On discontinuing treatment the obstruction returned rapidly in many patients These results are shown in Fig 2 A slight decrease in the amount of nasal mucus was seen initially but later no change was observed

Fig 3 shows the effect of Bdp on the nasal mucosa Minor signs of atrophy were present in a few patients before their inclusion in the preceding short term trial and in this trial There was no deterioration during this study

Purulent secretions noted in a few patients

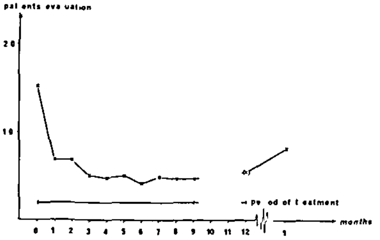


Fig 5 Patients evaluation of beclomethasone d prop onate treatment as estimated from weekly diaries Groups treated as stated in Figs 1–4

at the beginning of treatment showed a tendency to improve (Fig 4) The incidence of coryza did not increase during the study and some patients claimed that the purulent secretions which commonly follow a coryza cleared more quickly whilst they were on Bdp The patients diaries show a steady reduction of symptoms for the first 3 months of the study The clinical effect then remained constant until the treatment was discontinued (Fig 5) In 18 of 30 patients no treatment was necessary in the first three post trial months but 12 patients asked for re institution of treatment within the first few weeks after medication had been stopped An over all assessment of the efficacy of the treatment by the patients is given in Table I This assessment was in agreement with the evaluation based on rhinoscopy Twenty six of the 33 patients were treated with Bdp only but in seven patients this did not suffice Two of the latter patients had total blockage of the nose and polypectomies were required to permit entry of the Bdp One patient required 3 polypectomies during the study and one free of symptoms for the first 10 months of the study acquired an upper airway infection which caused rapid proliferation of his polyps An ethmoidectomy was required for clearance In 3 patients Bdp at the prescribed dosage had no effect and treatment was stopped X rays of the sinus were abnormal in all patients and remained unchanged during treatment There was no change in the bacterial flora of the nose when examined at the end of treatment and 30 days later Pathogenic bacteria were found in 5 out of 30 patients which compares favourably with an incidence of 7/45 in a matched control group Fungi identified as candida albicans were found in two patients but there were no signs or symptoms of candidiasis

Table I *Patients evaluation*

Excellent result	16
Good effect	11
Periodically good effect	2
Moderate effect	1
No effect	3
	33

No adverse reactions of any importance were observed On questioning 12 patients reported blowing blood stained mucus from the nose at the beginning of treatment There was no frank bleeding Three patients had a single crust in the anterior part of the nose which disappeared after the end of treatment Two patients complained of transient dryness of the nose In 19 patients no complaints could be elicited

CONCLUSION

It has been shown that local corticosteroid treatment of nasal polyposis gives a good or

excellent result in about 80% of patients It may be possible for patients so treated to avoid surgical removal of nasal polyps Intra nasal application of 400 μg Bdp daily seems to be close to an ideal basic treatment, but it must obviously be supplemented by surgery when the polyps are of such a size that the steroid cannot reach the mucous membrane Owing to the profound biological activity of Bdp, regular rhinoscopy is advised during long term treatment because of the possibility of local adverse reactions not yet recognized

ACKNOWLEDGEMENT

The beclomethasone dipropionate aerosol (Becotide Inhaler®) used in this investigation was supplied by Glaxo Denmark

ZUSAMMENFASSUNG

Doppelblindversuche von Patienten mit nasalen Polypen zeigten einen gunstigen Wirkungseffekt des Beclomethasone dipropionate Dieser Effekt wurde unter Anwendung von Langzeittherapie an 33 Nasenpolypose Patienten die durchschnittlich durch 15 Jahre Symptome zeigte uber pruft Der Grundriß des Untersuchungsprogrammes wird angegeben In 80% der Falle erreichte man ein ausgezeichnetes oder gutes Wirkungsresultat die Symptome betreffend nasale Sekretion nasale Obstruktion und Niesen Eventuelles Vorkommen von Nebeneffekten wurde klinisch und histologisch untersucht Man konstatierte ausschließlich leichte Blutungen zu Therapiebeginn Lokaltherapie mit Beclomethasone dipropionate wird fur die Nasenhohle als ideale Basistherapie für Patienten mit Nasenpolypen vorgeschlagen

REFERENCES

Mygind N Pedersen C B Prytz S & Sørensen H 1975 Treatment of nasal polyps with intranasal beclomethasone dipropionate aerosol *Clinical Allergy 5* 159

Sørensen H Mygind N Pedersen C B & Prytz S 1976 Long term treatment of nasal polyps with beclomethasone dipropionate aerosol III Morphological studies and conclusions *Acta Otolaryngol* (Stockh) *82* 260

Acta Otolaryngol 82 260–262, 1976

LONG-TERM TREATMENT OF NASAL POLYPS WITH BECLOMETHASONE DIPROPIONATE AEROSOL

III *Morphological Studies and Conclusions*

H Sørensen, N Mygind, C B Pedersen and S Prytz

From the University ENT Clinics Kommunehospitalet and Rigshospitalet Copenhagen Denmark

Abstract Morphological studies during the treatment of patients with nasal polyps with beclomethasone dipropionate aerosol for one year showed a trend towards a decrease in the number of eosinophils in nasal smears and falls in the increased concentrations of albumin IgG and IgE in the nasal secretion No change in the histological picture of the polypous tissue except increased density was observed By scanning electron microscopy no change in the surface structure of the nasal mucous membrane was noted As morphological studies did not reveal any sign of adverse effects beclomethasone dipropionate may be regarded as a valuable drug in the treatment of nasal polyps The indications for its clinical use and supplementary therapy are discussed

The therapeutic effect of beclomethasone dipropionate (Bdp) on nasal polyps has been demonstrated in a double blind investigation and in a long term clinical trial (Mygind et al , 1975 Pedersen et al 1975) In order to add to our knowledge of the action of the drug on the nasal mucous membrane the following studies were performed in 33 patients during treatment

1 Eosinophil counts in nasal smears
2 Quantitative measurements of the immunoglobulins in the nasal secretion
3 Histological studies of the polypous tissue
4 Scanning electron microscopy (SEM) of the surface of the nasal mucous membrane

Cytology

An increase in the eosinophil count of nasal smears is generally found in patients with nasal polyposis As only semi quantitative estimations of the number of cells can be obtained, the results are expressed as −, +, ++, +++ A decrease in the number of cells was found after three weeks treatment with Bdp This change did not reach statistical significance A similar result was observed in patients with perennial rhinitis on the same treatment (Wihl & Malm 1975) The result may be real, reflecting a reduced tendency of the eosinophils to pass through epithelium exposed to Bdp

Immunoglobulins

The concentrations of albumin IgG and IgE in nasal secretions are greatly increased in patients with nasal polyps (Table I) This is due to enhancement of the passage of these proteins from the vessels through the interstitial tissue into the secretion In the case of IgE and probably also IgG, local production is also increased (Mygind et al , 1975*b*) During treatment, the concentrations of these proteins decline towards normal IgA (mainly secretory IgA) does not vary with the other proteins and its concentration does not alter during treatment with Bdp

Histology

The histological picture of the polypous tissue was examined before, during and after treatment A biopsy specimen was taken from

Table I *Concentration of proteins in nasal secretion from patients with nasal polyps before and after long term treatment with beclomethasone dipropionate aerosol compared with matched normal control persons*

		No of patients	Median (mg/100 ml)	25–75 percentile (mg/100 ml)	Mann-Whitney rank sum test
Albumin	Before treatment	12	450	130–920	$p<0.01$
	After treatment	20	124	67–720	
	Normal controls	29	56	45–120	
IgG	Before treatment	14	180	57–255	$p<0.01$; before vs after $p<0.05$
	After treatment	19	38	18– 89	
	Normal controls	29	28	15– 46	
IgA	Before treatment	13	75	28–165	No significant differences
	After treatment	21	73	35–283	
	Normal controls	29	78	58–118	
IgE	Before treatment	16	68[a]	31–366	$p<0.01$
	After treatment	19	21	12–125	
	Normal controls	33	(5)	<5– 12	

[a] All IgE values are units/ml
For methods see Mygind et al 1975*b*

a polyp in each patient without analgesia More than 100 specimens were examined and the histological evaluation, partly quantitative, was carried out blindly to exclude bias The only significant change that could be observed was an increased density of the interstitial tissue due to a reduction of the intercellular fluid The number of eosinophils in the tissue was unchanged No systematic change in the distribution of pseudostratified ciliated and squamous epithelium was recorded during treatment

Scanning Electron Microscopy

This investigation was carried out on a blind basis, 40 biopsy specimens from these patients were examined

The epithelial surface of the nasal polyps fall into four categories (1) Typical respiratory epithelium densely covered by cilia (2) Transitional type with microvilli (3) Squamous epithelium without microvilli (4) Horny squamous epithelium with desquamation

No tendency either towards respiratory epithelium or towards squamous epithelium was observed during treatment The surface of the nasal mucous membrane showed no morphological change during local corticosteroid application Particulars of the histological and SEM investigations will be reported in a separate paper elsewhere (Sørensen et al)

The results of the morphological and immunological investigations can be summarized as follows

1 Nasal polyposis causes marked pathological changes, such as local eosinophilia, oedema of the mucous membrane and an increase of plasma proteins in the nasal secretions

2 During treatment with Bdp a normalizing effect was noticed The permeability of blood vessels was reduced, thus decreasing oedema as well as the concentration of proteins in the nasal secretion

3 Tissue eosinophilia was not influenced by treatment and eosinophilia in the secretion only to a slight degree Although the treatment markedly reduced or eradicated the subjective symptoms, the mucous membrane did not return to normal

4 Treatment did not influence local IgA production, but further investigations are required to elucidate the effects of Bdp on this immune system

5 After one year's treatment no tendency towards squamous metaplasia, characteristic of atrophic rhinitis, was found

CONCLUSION

A 3-week double blind investigation showed that a fair therapeutic effect could be obtained in patients with nasal polyps with intranasal Bdp aerosol (dose 400 μg/day) In a long term clinical investigation (9–12 months) it ap peared that continuous treatment controlled the symptoms in the majority of patients and had no adverse clinical or histological effects Thus, Bdp can be regarded as a valuable drug in the treatment of nasal polyps

Bdp is not suitable in all cases of nasal polyps In patients with small, slowly expand ing polyps the treatment of choice is probably surgical removal

In patients with more pronounced symp toms or rapid recurrence after extirpation Bdp treatment is indicated In such cases, Bdp should be used as a basic treatment If necessary, it may be supported by surgery, systemic corticosteroids or antibiotics, de pending on the nature and severity of symp toms When total blockage occurs, partial polypectomy from the lower anterior part of the nose is indicated to allow penetration by the aerosol In some cases systemic cortico- steroid should be administered initially to re duce the size of the polyps, thus permitting Bdp to enter the cavity

Resistance to treatment with Bdp may be due to

1 Total blockage of the nasal cavity, which prevents penetration of the drug (surgical re moval necessary)

2 Infection of the nasal mucous membrane (antibiotics may be indicated)

3 Rapid growth (systemic corticosteroid may support the effect of treatment)

4 Involvement of the sinuses accounting for the major part of the symptoms (surgical operation with clearance of the affected sinuses)

It should be stressed that Bdp treatment is symptomatic only After stopping treatment polyps will recur in many patients However intermittent treatment with Bdp will enable the majority of patients to live a normal life It is important that any patient treated with Bdp should be seen at regular intervals, as any ef fective treatment of nasal polyps may lead to atrophy of the mucous membrane, secondary to increased space in the nasal cavity In our studies, no sign of atrophy attributable to Bdp was observed

ZUSAMMENFASSUNG

Histologische Untersuchung nasaler Abstrichpräparate von Patienten mit Nasalpolypen unter Beclomethasone dipropionate Arosolbehandlung zeigten Tendenz zur Abnahme der Anzahl von eosinophilen Leukocyten und Fall der erhöhten Albuminkonzentration IgG IgE im Nasen sekret Das histologische Bild ist unverandert abgesehen von Zunahme der Dichte des polyposen Gewebes Elektronmikroskopische Untersuchungen ergaben keinerlei Veranderungen in der Oberflächenstruktur der nasalen Mucosa Die morphologischen Untersuchungen erbrachten keine Zeichen eines ungunstigen Effektes Beclomethasone dipropionate wird als eine wertvolle Droge in der Behandlung nasaler Polypen angesehen Es wird das klinische Indikationsgebiet im Vergleich mit anderen Behandlungsmethoden diskutiert

REFERENCES

Mygind N Pedersen C B Prytz S & Sørensen H 1975*a* Treatment of nasal polyps with intranasal beclomethasone dipropionate aerosol *Clinical Allergy 5* 159

Mygind N Weeke B & Ullman S 1975*b* Quantitative determination of immunoglobulins in nasal secretion *Int Archs Allergy Appl Immunol 49* 99

Pedersen C B Mygind N Sørensen H & Prytz S 1976 Long term treatment of nasal polyps with beclomethasone dipropionate aerosol II Clinical results *Acta Otolaryngol* (Stockh) *82* 256

Sørensen H Mygind N Pedersen C B & Prytz S The nasal mucosa during long term treatment with beclomethasone dipropionate aerosol A light and scanning electron microscopic study In preparation

Wihl J Å & Malm L 1975 Personal communication

Acta Otolaryngol 82 263–265, 1976

NASAL CANCER AND NASAL MUCUS-TRANSPORT RATES IN WOODWORKERS

H C Andersen,[1] J Solgaard[2] and I Andersen[2]

From the [1]ENT Department and the [2]Institute of Hygiene University of Aarhus Aarhus Denmark

Abstract Among 17 patients with adenocarcinoma of the nasal cavity 12 had been heavily exposed to wood-dust inhalation 10 as furniture makers one as a turner and one as a coach builder Measurements of the nasal mucus transport rate in 68 wood-dust workers demonstrated a significantly higher frequency of mucostasis than in a control group

British investigators (Acheson et al, 1968) have drawn attention to inhalation of wood-dust as a potential source of adenocarcinoma of the nasal cavity They found that the incidence of this tumour among furniture makers in the Oxford area was 0 7 per 1 000, as compared with the incidence of all kinds of malignancies involving the nasal cavity and nasal sinuses, which was 11 2 per million in the general population They demonstrated that the latency period for tumour development was extremely long, viz 30–50 years, and that exposure-free periods in persons who had for a long time been exposed to wood-dust did not prevent the development of adenocarcinoma

On the basis of these observations, adenocarcinoma of the nasal cavity in furniture makers is now accepted as an occupational disease in Great Britain

Similar observations have been made in France and Belgium

A survey of the occupational distribution of patients with carcinoma of the nose and paranasal sinuses treated in the ENT Department and the Radium Centre of the University Hospital, Aarhus, within the decade 1964–74 comprises a total of 157 cases

From Tables I and II it is seen that the adenocarcinomata were exclusively located in the nasal cavity, and that a large majority of the patients with this tumour type had been exposed to wood-dust inhalation

Table I

Ectodermal tumours	Vestibule	Nasal cavity	Maxillary sinus	Total	Wood-dust exposure
Epithelial papilloma		33		33	1
Epidermoid carcinoma	26	16	29	71	5
Adenocarcinoma		17		17	12
Adeno-cyst carc		4	5	9	1
Malign sialoma			1	1	
Transit carcinoma		3	1	4	
Anaplast carcinoma	1	7	1	9	1
Malign melanoma		13		13	2
Total	27	93	37	157	22

5 After one year's treatment no tendency towards squamous metaplasia, characteristic of atrophic rhinitis, was found

CONCLUSION

A 3 week double blind investigation showed that a fair therapeutic effect could be obtained in patients with nasal polyps with intranasal Bdp aerosol (dose 400 μg/day) In a long term clinical investigation (9–12 months) it appeared that continuous treatment controlled the symptoms in the majority of patients and had no adverse clinical or histological effects Thus, Bdp can be regarded as a valuable drug in the treatment of nasal polyps

Bdp is not suitable in all cases of nasal polyps In patients with small, slowly expanding polyps the treatment of choice is probably surgical removal

In patients with more pronounced symptoms or rapid recurrence after extirpation Bdp treatment is indicated In such cases, Bdp should be used as a basic treatment If necessary, it may be supported by surgery, systemic corticosteroids or antibiotics, depending on the nature and severity of symptoms When total blockage occurs partial polypectomy from the lower anterior part of the nose is indicated to allow penetration by the aerosol In some cases, systemic corticosteroid should be administered initially to reduce the size of the polyps, thus permitting Bdp to enter the cavity

Resistance to treatment with Bdp may be due to

1 Total blockage of the nasal cavity, which prevents penetration of the drug (surgical removal necessary)

2 Infection of the nasal mucous membrane (antibiotics may be indicated)

3 Rapid growth (systemic corticosteroid may support the effect of treatment)

4 Involvement of the sinuses accounting for the major part of the symptoms (surgical operation with clearance of the affected sinuses)

It should be stressed that Bdp treatment is symptomatic only After stopping treatment polyps will recur in many patients However intermittent treatment with Bdp will enable the majority of patients to live a normal life It is important that any patient treated with Bdp should be seen at regular intervals as any effective treatment of nasal polyps may lead to atrophy of the mucous membrane, secondary to increased space in the nasal cavity In our studies, no sign of atrophy attributable to Bdp was observed

ZUSAMMENFASSUNG

Histologische Untersuchung nasaler Abstrichpraparate von Patienten mit Nasalpolypen unter Beclomethasone dipropionate Arosolbehandlung zeigten Tendenz zur Abnahme der Anzahl von eosinophilen Leukocyten und Fall der erhohten Albuminkonzentration IgG IgE im Nasensekret Das histologische Bild ist unverandert abgesehen von Zunahme der Dichte des polyposen Gewebes Elektronmikroskopische Untersuchungen ergaben keinerlei Veranderungen in der Oberflachenstruktur der nasalen Mucosa Die morphologischen Untersuchungen erbrachten keine Zeichen eines ungunstigen Effektes Beclomethasone dipropionate wird als eine wertvolle Droge in der Behandlung nasaler Polypen angesehen Es wird das klinische Indikationsgebiet im Vergleich mit anderen Behandlungsmethoden diskutiert

REFERENCES

159

Mygind N Weeke B & Ullman S 1975*b* Quantitative determination of immunoglobulins in nasal secretion *Int Archs Allergy Appl Immunol* 49 99

Pedersen C B Mygind N Sørensen H & Prytz S 1976 Long term treatment of nasal polyps with beclomethasone dipropionate aerosol II Clinical results *Acta Otolaryngol* (Stockh) 82 256

Sørensen H Mygind N Pedersen C B & Prytz S The nasal mucosa during long term treatment with beclomethasone dipropionate aerosol A light and scanning electron microscopic study In preparation

Wihl J Å & Malm L 1975 Personal communication

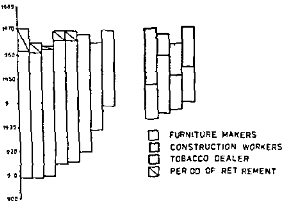


Fig 1

taste appears and the dye is seen by pharyngeal inspection is measured (Andersen et al, 1974) The transport time is inversely proportional to the fastest nasal mucus transport rate Of the woodworkers, 38% had mucostasis (transport time >30 min), as compared with only 14% in a control group of 66 subjects with no occupational dust exposure

Simultaneous measurements of the dust concentration in the breathing zone of the workers were carried out with a portable pump and filter system The dust samples were measured gravimetrically and by microscopy The size distribution was found to have a maximum at 5–10 μm

The dust concentration was higher than the threshold limit value (TLV) of 5 mg wood dust/m^3 air in 63% of the measurements and higher than 10 mg/m^3 in 28%

The number of woodworkers with nasal mucostasis was directly proportional to the wood-dust concentration

For a detailed report of this investigation and a comprehensive list of references, see Solgaard & Andersen (1975) and Andersen (1975)

ZUSAMMENFASSUNG

Von 17 Patienten mit Adenokarzinom in der Nasenhohle waren 12 viele Jahre hindurch einem massiven Einatmen von Holzstaub ausgesetzt gewesen Zehn derselben hat ten in der Möbelindustrie gearbeitet Eine Untersuchung des nasalen Schleimtransportes bei 68 in der Mobelindustrie tatigen Personen ergab daß die Anzahl Falle von Schleimstauung in dieser Gruppe signifikant großer war als in einer Kontrollgruppe

List of references may be requested from the authors

Acta Otolaryngol 82 268–270 1976

AUDIOLOGICAL TREATMENT OF PRESCHOOL CHILDREN WITH HEARING IMPAIRMENTS

H F Fabritius, V Gaustad and A Kallset

From the Department of Audiology Namdal Hospital Namsos and Vikhov School for the Hard of Hearing Vikhamar Norway

Abstract A system by which children with impaired hearing are given early audiological treatment is reported The treatment has been carried out during short admissions to institution alternating with stays at home Special training and instruction of the parents are important The authors are of the opinion that this type of preschool training is suitable for regions with scattered population Even a small institution with a limited staff can give treatment to a considerable number of children

The importance of the early audiological treatment of children with severe hearing impairments is generally recognized In cities and urban districts this treatment can nowadays easily be given without admitting the child to n institution But in districts with a scattered population and great travelling distances—as it is the case in many parts of Norway—other means of audiological treatment must be used Here a temporary admission to an institution will be necessary

In Norway we have three centres where audiological treatment in institution alternates with stays at home combined with instruction of the parents These centres are located in Ålesund Gjovik and Namsos They vary a little according to the density of population and geographical location

We shall give a brief survey of this work in Namsos and present some of our experiences, with reference to the medical psychological and educational points of view

In connection with the ENT Department in Namsos there is a small preschool institution called the Hearing Annex The children are received at the H A immediately when their handicap is recorded If the child is younger than 3 years, it is always admitted together with one of the parents The stay in the institution lasts only 1 week, but is repeated at intervals of some weeks

This first admission period is used mainly to inform and instruct the parents as to how to help the child in lipreading and auditory training, as well as in the use of various technical facilities, such as hearing aids and speech amplifiers The child is trained to adapt socially Gradually some individual guidance is introduced We regard these stays in the H A at an early age as a valuable introduction to more permanent institutional life later after the age of 3 years In the institution the children are divided into groups of 5 or 6 according to age, speech level and ability to adapt socially Each group stays 3 weeks in the H A , twice in the autumn and three times in the spring One of the parents will always accompany the child and bring it back home This gives the parents an opportunity to establish a most important contact with the individual members of the staff

During the 'stay at home' period the parents—usually the mother—receives a work or training programme which is revised and discussed when a new institutional stay starts

Table I

Schools	Number of children	Average hearing loss
Regular school	4	Right ear 76 dB left ear 80 dB
School for the hard-of hearing	11	Right ear, 93 dB left ear, 87 dB
School for the deaf	8	Right ear, 103 dB left ear 108 dB

Every third week there is a staff meeting in the H A The teachers, the head of the toy library, the psychologist, the peripatetic teacher and the doctors discuss the children's situation and status and the further programme

In the northern part of Norway there are few facilities for children with impaired hearing As a consequence, the H A in Namsos serves children from a large region They come from the five northernmost counties in Norway

The H A has been in operation for 7 years, and we find it is appropriate to give a survey of the results The children are admitted to the H A at different age levels, depending upon the standard of diagnostic facilities and hearing treatment in their home district Some of them have been admitted rather late Consequently, the audiological treatment in the H A has been of different duration The minimum treatment in this series has been five admissions during 2 years and the maximum 20 admissions during 5½ years

Twenty-three children had most of their preschool training in the H A. Afterwards they had at least one year in a regular school or in a special school Of the 23 children, 4 are in regular school, 11 in schools for the hard-of-hearing and 8 in the school for the deaf The series of children is presented in Table I

The causes of impaired hearing were Rubella, 11, erythroblastosis, 1, toxic otitis, 1, meningitis, 3, unknown, 5 Rubella, as the cause of impaired hearing in 11 out of 23 children is a highly important factor, although it does not have general significance

Table II

Level of mental ability	Girls	Boys	Total
Mental ability far below average for the age group IQ<75	0	0	0
Mental ability somewhat below average for the age group IQ 75–90	0	0	0
Mental ability equivalent to normal age group IQ 90–110	8	4	12
Mental ability somewhat above average for the age group IQ 110–125	2	3	5
Mental ability far above average for the age group IQ >125	4	0	4

Psychological evaluation

Psychological evaluation and intelligence tests have been performed in 21 of the 23 children The tests have been carried out by five different psychologists but most of the tests have been done by one of them She has also made the total evaluation Mainly non-verbal tests are used A rough classification has been made according to Table II

More than one-third of the children showed fluctuation in mental performance Since non-verbal tests are used, the results of the tests and the child s intelligence cannot be directly compared The psychologist is more inclined to regard the results as a measure of the child s total intellectual resources, which cor-

Table III *Evaluation of 4 children in regular school*

	Good	Medium	Below medium
Speech development	1	3	0
Social adaptability	4	0	0
Professional status	1	2	1
Able to use a hearing aid	4		

Acta Otolaryngol 82 268-270, 1976

AUDIOLOGICAL TREATMENT OF PRESCHOOL CHILDREN WITH HEARING IMPAIRMENTS

H F Fabritius, V Gaustad and A Kallset

From the Department of Audiology Namdal Hospital Namsos and Vikhov School for the Hard of Hearing Vikhamar Norway

Abstract A system by which children with impaired hearing are given early audiological treatment is reported The treatment has been carried out during short admissions to institution alternating with stays at home Special training and instruction of the parents are important The authors are of the opinion that this type of preschool training is suitable for regions with scattered population Even *a small institution with a limited staff can give treatment* to a considerable number of children

The importance of the early audiological treatment of children with severe hearing impairments is generally recognized In cities and urban districts this treatment can nowadays easily be given, without admitting the child to institution But in districts with a scattered population and great travelling distances—as it is the case in many parts of Norway—other means of audiological treatment must be used *Here a temporary admission to an institution* will be necessary

In Norway we have three centres where audiological treatment in institution alternates with stays at home combined with instruction of the parents These centres are located in Ålesund, Gjovik and Namsos They vary a little according to the density of population and geographical location

We shall give a brief survey of this work in Namsos and present some of our experiences, with reference to the medical psychological and educational points of view

In connection with *the ENT Department* in Namsos there is a small preschool institution called the Hearing Annex The children are received at the H A immediately when their handicap is recorded If the child is younger than 3 years, it is always admitted together with one of the parents The stay in the institution lasts only 1 week, but is repeated at intervals of some weeks

This first admission period is used mainly to inform and instruct the parents as to how to help the child in lipreading and auditory training, as well as in the use of various technical facilities, such as hearing aids and speech amplifiers The child is trained to adapt socially Gradually some individual guidance is introduced We regard these stays in the H A at an early age as a valuable introduction to more permanent institutional life later *after the age of 3 years In the institution* the children are divided into groups of 5 or 6 according to age, speech level and ability to adapt socially Each group stays 3 weeks in the H A , twice in the autumn and three times in the spring One of the parents will always accompany the child and bring it back home This gives the parents an opportunity to establish a most important contact with the individual members of the staff

During the "stay at home period the parents—usually the mother—receives a work or training programme which is revised and *discussed when a new institutional stay starts*

Acta Otolaryngol 82 271–274, 1976

SJÖGREN'S SYNDROME

J Johansen

From the Department of Otolaryngology Rikshospitalet University Hospital Oslo Norway

Abstract Sjogren s syndrome is a multisystemic connective tissue disease The syndrome is associated with other major connective tissue disorders such as rheumatoid arthritis, systemic lupus erythematosus systemic sclerosis and others Reticulo-endothelial malignances have repeatedly been reported in patients with Sjogren s syndrome A clinical study of 20 patients with Sjogren s syndrome was performed in the Department of otolaryngology, Rikshospitalet, Oslo Norway A considerable complexity of symptoms was seen One patient with malignant lymphoma and one with a thyroid carcinoma were encountered

In 1936, von Grosz introduced the term Sjögren's syndrome to honour the Swedish ophthalmologist Henrik Sjögren for his work of keratoconjunctivitis sicca, parotid enlargements with xerostomia and rheumatoid arthritis, all components of a generalized symptom complex

Aetiology

The aetiology of Sjogren s syndrome is unknown The remarkable degree of immunological abnormalities hypergammaglobulinaemia, rheumatoid factor, antinuclear anti bodies, and antibodies to extracts from salivary and lacrimal glands, might indicate an auto-immune disease Identification of the antigen and demonstration of the antibody's specificity for the antigen has not been achieved In the last decade, attention has been directed to the theory of slow virus as a causative agent The speculations are based upon the animal models of NZB/NZW mice They develop after the age of four months pathological changes analogous to Sjögren's syndrome (Kessler, 1968)

Virus like particles resembling the type C oncogenic virus are identified by electron microscopy The tissue damage observed in Sjögren's syndrome could be explained as the result of direct invasion by the infectious agent, or as resulting in part from autoimmune mechanisms, especially immune complex deposition on vascular structures (Shearn, 1971)

Diagnosis

The diagnosis of Sjögren's syndrome is based upon the clinical appearance, laboratory findings and ocular and salivary gland tests

About 90% of the patients are women aged between 40 and 60 years Laboratory features are immunological abnormalities, elevated SR and moderate anaemia, normochromic or hypochromic Ocular tests are the Schirmer tear tests I and II, measuring the tear flow, and Rose bengal staining test showing devitalized tissue of the conjunctiva and cornea

Ocular complications may be seen, such as corneal ulcer, nodular episcleritis, uveitis and posterior subcapsular cataracts In the animal mode, deeper damage in the eye is frequent Sialograms of the patient with Sjogren's syndrome are abnormal Rubin & Holt (1957) divided them into four stages punctate, globular cavitary and destructive, according to the progressive increase in abnormalities

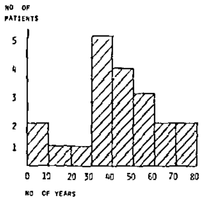


Fig 1 The age distribution of the patients

Radio isotope studies with (^{99m}TC) demon strate in the scintisan the functional condition of the gland

Histopathological examination of biopsy specimens from the major salivary glands show lymphoid infiltration within the lobules, subsequent atrophy and loss of acinar tissue, hyperplasia of ductal epithelium forming myo epithelial islands In the minor salivary glands the last characteristic is missing

Treatment

Treatment of Sjogren's syndrome is sympto matic The decrease in tears can be corrected by artificial tears from a reservoir of wetting solutions on glasses Xerostomia, dysphagia and dental caries are treated by oral lubrica tion and high water intake, careful dental hygiene and fluorine locally for the teeth.

Prognosis

Prognosis in Sjogren s syndrome is related to the severity of the associated connective tissue disease Death rates in patients with Sjogren's syndrome are not available A high incidence of infection and potential malignant lymphoma may give an increased risk of early death, even without associated connective tissue disease

Table I *Initial symptoms in the patients and their further development during the observa tion period*

	%
Salivary gland disorder	70–100
Rheumatic disorder	15– 30
Keratoconjunctivitis	10– 80
Thyroid disease	5– 5

Table II *Results of laboratory tests*

	No of patients tested	Abnormal n	Abnormal %
Immunological abnormalities			
Hypergammaglobulinaemia	20	13	65
Hyperimmunglobulinaemia	20	13	65
Haematological abnormalities			
Elevated SR	20	11	55
Anaemia	20	4	20

MATERIAL AND METHODS

The present study was performed in 20 pa tients with Sjogren's syndrome selected from a group of 150 patients referred to the Depart ment of Otolaryngology, Rikshospitalet Oslo Norway, during the period 1960–1974 The pa tients were 17 women and 3 men The age distribution, periods of observation and initial symptoms followed during observation are shown in Figs 1, 2, and Table I

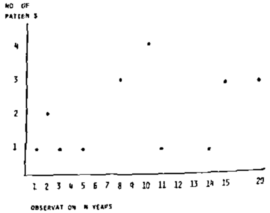


Fig 2 Periods of observation

Table III *Results of special organ tests*

		n	%
Salivary gland examinations			
Sialography	15	14	93
Radio-isotope test	15	15	100
Histology	13	13	100
Ocular tests			
Rose bengal	16	11	68
Schirmer	16	8	50

In this selected group there is a natural preponderance of symptoms from the salivary glands In most patients, the disease started with recurrent swellings of the parotid gland During the observation period xerostomia developed in all patients The odontogenic status was extremely poor, except for some patients with mild symptoms The majority of patients were prosthesis carriers The rheumatic group includes patients with rheumatoid arthritis and degenerative joint disease not based on the New York criteria

The symptoms of keratoconjunctivitis is based upon anamnestic information later confirmed by ocular tests The thyroid disorder was carcinoma of the thyroid in a patient with severe xerostomia and severe xerophtalmus

RESULTS

Tables II and III show the results of laboratory tests, salivary gland examinations and ocular tests The findings are similar to those expected from empirical data of other reports The patients not examined had mild forms of Sjogren's syndrome and were not yet expected to benefit of further examinations

DISCUSSION

Table IV *might serve as a basis for the discussion* of the study The series is divided in three main groups mild, moderate and severe forms of Sjogren's syndrome Need of medical treatment and working ability served as parameters for grading the material In mild forms the patients possessed full working ability and only occasionally needed medical treatment In moderate forms, the working ability was diminished and medical treatment needed In severe forms, the prognosis was poor, no working ability and urgent need for medical treatment In these groups, we found a thyroid carcinoma, a lymphosarcoma and a patient with five cardial infarctions and pericorditis

We learn from this study that mild forms of Sjogren's syndrome do not necessarily develop into more severe forms Moderate forms will often remain moderate for a long time The crucial point seems to be the associated connective tissue disorders Even in mild forms we find them, their course is unpredictable

This arranging of the material following descriptive statistical terms has no statistical validity because it is built upon a selected material It might, however, serve as a guidance in gaining information on Sjogren's syndrome

Table IV *The course of disease in various patient groups with results of tests*

	Form		Age	Duration	Gamma globulinopathia		Associated connective tissue disease	
	n	%	*n*	*n*	*n*	%	*n*	%
Mild	5	(25)	25–83	1–25	0	(0)	2	(40)
Moderate	12	(60)	24–79	2–25	10	(83)	3	(25)
Severe	3	(15)	11–71	2– 3	3	(100)	3	(100)

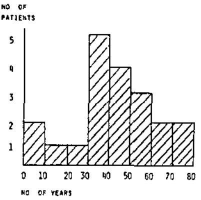


Fig 1 The age distribution of the patients

Radio isotope studies with (^{99m}TC) demonstrate in the scintisan the functional condition of the gland

Histopathological examination of biopsy specimens from the major salivary glands show lymphoid infiltration within the lobules, subsequent atrophy and loss of acinar tissue, hyperplasia of ductal epithelium forming myo epithelial islands In the minor salivary glands the last characteristic is missing

Treatment

Treatment of Sjogren's syndrome is sympto matic The decrease in tears can be corrected by artificial tears from a reservoir of wetting [illegible] on glasses Xerostomia, dysphagia [illegible]d dental caries are treated by oral lubrica tion and high water intake, careful dental hygiene and fluorine locally for the teeth

Prognosis

Prognosis in Sjogren's syndrome is related to the severity of the associated connective tissue disease Death rates in patients with Sjogren's syndrome are not available A high incidence of infection and potential malignant lymphoma may give an increased risk of early death, even without associated connective tissue disease

Table I *Initial symptoms in the patients and their further development during the observa tion period*

	%
Salivary gland disorder	70–100
Rheumatic disorder	15– 30
Keratoconjunctivitis	10– 80
Thyroid disease	5– 5

Table II *Results of laboratory tests*

	No of patients tested	Abnormal *n*	Abnormal %
Immunological abnormalities			
Hypergammaglobulinaemia	20	13	65
Hyperimmunglobulinaemia	20	13	65
Haematological abnormalities			
Elevated SR	20	11	55
Anaemia	20	4	20

MATERIAL AND METHODS

The present study was performed in 20 pa tients with Sjogren's syndrome selected from a group of 150 patients referred to the Depart ment of Otolaryngology, Rikshospitalet, Oslo, Norway, during the period 1960–1974 The pa tients were 17 women and 3 men The age distribution, periods of observation and initial symptoms followed during observation are shown in Figs 1, 2, and Table I

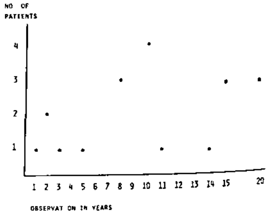


Fig 2 Periods of observation

Acta Otolaryngol 82 275–278, 1976

NOSOCOMIAL PAROTITIS

A Lundgren, P Kylen and L M Odkvist

From the ENT Clinic Regional and University Hospital Linkoping Sweden

Abstract A total of 22 cases of acute suppurative parotitis are reviewed The causative factors were severe primary disease with salivary gland hyposecretion related to age (77 years) dehydration oral inactivity and drugs (19 patients) The oral hygiene was poor and permitted ascending canalicular invasion of resistant staphylococci The mortality was 27% The treatment including preferably cloxacilline and incision is discussed To stress the importance of prophylaxis the name nosocomial parotitis is suggested

Acute suppurative parotitis usually occurs in elderly debilitated patients who are seriously ill Some commonly used synonyms are acute postoperative parotitis, acute necrotic parotitis, acute gangrenous parotitis, acute necrotic parotitis, septic parotitis and parotid abscess (Hemenway & English, 1971) A study of a group of patients suffering from acute suppurative parotitis treated at the ENT Clinic of the Regional and University Hospital, Linkoping is presented A review of the symptomatology pathogenesis and treatment as well as a reason for the name ' nosocomial parotitis are given

MATERIAL AND METHOD

Twenty two cases of acute suppurative salivary gland inflammation have been treated The patients were referred for consultation from various departments in the hospital or were treated in the wards of the ENT Clinic Seventeen of the cases had a unilateral parotitis, three cases were bilateral Two cases of a similar inflammation of the submandibular gland have been included All of the patients had a serious primary disease In addition, several of the patients were also debilitated due to advanced age The average age was 77 years (range 60–92) Nine were men and thirteen women

In seven cases the inflammation followed an operation At the time of onset the patients were on parenteral nutrition (10 cases), diuretic medication (8 cases), psychotrophic drugs of different types given because of agitation anxiety insomnia or more serious mental disturbances (10 cases), or atropine and anticholinergic drugs (8 cases) Only three patients were free from drugs (Table 1)

Twelve of the patients had been treated with antibiotics Bacterial cultures in 19 of the cases showed *Staphylococcus aureus* which was resistant to most antibiotics, but without exception sensitive to cloxacillin, also mostly to chloramphenicol and erythromycin (Fig 1) The phage-typing usually showed strain 80/81, the nosocomial type With a few exceptions all cases were treated with large doses of cloxacilline A few early cases were operated on under general anaesthesia, with parotidectomy skin incisions and broad incisions of the parotid fascia and extensive drainage of the parotid and paraparotid spaces as suggested by Perzik (1962) In later cases, we used the same principles of drainage, but

Table I *Various drugs presumably contributing to a low salivary flow*

Diur=diuretics Antichol=anticholinergics Psychotr=psychotropics Antihist=antihistaminics

Case	Diur	Antichol	Psychotr	Antihist
1	+		+++	
2			+	+
3			+++++	
4		+		
5		+		
6	+	+		
7		+	+	+
8	+			
9			+++	+
10		+	+	
11			++	
12		+		
13	+			
14		+	+	
15				
16	+		+	
17		+		
18	+		++	+
19	+			
20	+			
21				
22				

operated earlier under local anaesthesia and were thus able to make more limited incisions and drainage In some cases, repeated punctures of an abscess were sufficient This change towards a more conservative attitude was due to the fact that we now have better intensive care and better antibiotics Six patients (27%) died within 2 weeks and 9 patients (40%) within 2 months after the onset of parotitis The cause of death in the early cases was a combination of a primary disease and the extra load of the parotitis The late deaths were due to the primary disease, the parotitis being healed

DISCUSSION

A Symptoms

The typical initial symptoms of parotitis are moderate swelling of the parotid region with some tenderness and pain At this stage, the parotitis may heal spontaneously or within 1-7 days develop into a fully established suppurative parotitis with rapid swelling of the gland, with tense, shining red blue skin trismus, pain and intense local tenderness impeding free head and neck movements The patients have fever and are seriously affected by the disease An abscess may occur as may gangrene of the gland and fistulation through the overlying skin, to the external auditory meatus, the temporo mandibular joint or to the parapharyngeal regions with further progress to the mediastinum Secondary glottic oedema with respiratory stridor may occur (Laburthe Tolra, 1969) Almost exclusively, the parotid gland is affected rarely the submandibular gland Of our 22 patients, only 2 had a submandibular inflammation It is reported that the submandibular glands are so infrequently involved in this infection because of better protection by mucines (Rausch, 1959) In the 7 patients in whom parotitis developed after an operation the disease began 4 to 8 days postoperatively, which is in accordance with the literature (Perzik, 1962)

B Pathogenesis

1 *Primary disease*

Acute, suppurative parotitis occurs in elderly *debilitated patients who are seriously ill* often, but not necessarily, postoperatively

2 *Hyposecretory factors*

In the case history there are always factors contributing to salivary hyposecretion *Age* is one of the most important factors It has been shown that the secretion from the salivary glands decreases with advancing age (Wainwright & Becks, 1943, Korting & Kleinschmidt, 1953) In our series, the average age was 77 years It is well known that in cases of *dehydration* the salivary secretion is diminished, sometimes noticeable as xerostomia The salivary gland activity is stimulated by food intake, by smell and taste and partly by the mechanical effects of chewing and thus *oral inactivity* leads to salivary hyposecretion Xerostomia is often caused by *drugs* either by their dehydrating action or by actively

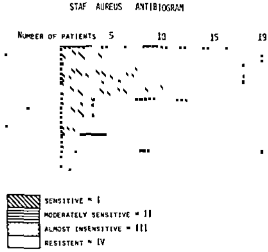


Fig 1 Antibiogram for the pathogenic staphylococci in 19 of the cases

decreasing the salivary flow These drugs are primarily diuretics, parasympatholytics barbiturates, tricyclic antidepressants and other psychotropic drugs and antihistamines

The majority of our patients were on drugs from the list above administered either singly or in combinations Only three of them were drug free at the onset of parotitis (Table I)

3 *Secondary bacterial infection or invasion*

Normally the saliva flushes the salivary duct system preventing infection When the salivary flow ceases or is diminished, bacteria from the oral cavity may in the presence of poor hygiene invade the parotid gland duct (Arnulf, 1951, Faye & Deffez, 1969)

Many other theories concerning enzymatic autodigestion reflexes from the abdominal organs toxic allergic influence and primary infection have been advanced as regards the cause of acute suppurative parotitis but they have not been conclusively proved

Treatment

The most important measure is to prevent the parotitis Patients in whom this complication is likely to occur must have a high fluid intake and any administration of salivation depressant drugs must be avoided The salivary secretion should be stimulated by lemon juice and by maintaining oral activity, for example, by using chewing gum The oral hygiene should not be neglected, the oral cavity should be washed and the teeth frequently cleaned Artificial dentures should not be worn Oral infections should be treated promptly The patients must be carefully observed for early symptoms of parotitis as treatment at this stage is highly effective In case of a manifest septic parotitis, this regimen should be supplemented by the administration of antibiotic drugs initially preferably cloxacillin An alternative antibiotic should be given only according to bacterial cultures and antibiograms (see Fig 1)

The diagnosis of a parotid abscess may be difficult as the tense parotid fascia obscures abscess fluctuations by the palpating hand Needle punctures of the parotid gland may in such cases give the diagnosis and the aspiration of pus accelerates healing, and provides material for a representative bacterial culture If the skin overlying the gland is hard, tense and red, incision through the skin should not be postponed, even if needle aspiration is negative A moderately long incision behind the mandibular angle under local anaesthesia and drainage of the parotid and paraparotid spaces are the procedures of choice Thorough knowledge of the anatomy prevents damage to the facial nerve Long incisions and explorations under general anaesthesia in these patients may be fatal if performed in weak patients Drainage tubes or successive debridements on consecutive days are necessary until healing is achieved Analgesic drugs are usually necessary

Prognosis

The appearance of acute suppurative parotitis is to be looked upon as an ominous sign It is obvious that the acute suppurative parotitis in these already severely ill patients may be

the additional complication that the patient cannot tolerate The cause of death is, however, most often the primary disease The mortality is approximately 5–35% (Rausch, 1959), in our series 27% within 2 weeks

CONCLUSIONS

Acute suppurative parotitis affects seriously ill, elderly patients with an age-related diminished salivary secretion The secretion is further diminished because of insufficient fluid intake, oral inactivity, and the presence of oral bacteria that may ascend through Stensen's duct to the parotid gland The organisms are most frequently resistant staphylococci We suggest the new provocative name "nosocomial parotitis" to stress that this illness will appear in hospitalized patients if we impair the salivary gland secretion and neglect the patient's oral and environmental hygiene

ZUSAMMENFASSUNG

22 Falle von akutem suppurativem Parotit sind bearbeitet worden Die Ursachen die zu diesem Zustand geführt haben sind schwere primare Krankheit mit altersbedingter Hyposekretion in den Speicheldrusen (Alter 77 Jahre) Dehydration orale Inaktivitat und Pharmaka (19 Patienten) Die orale Hygiene war schlecht und verursachte aufwartssteigende kanalikulare Invasion von Parotis mit *resistenten Staphylokokken Mortalitat 27% Die Behand*lung vor allem mit Cloxacillin und Inzision ist diskutiert worden Um die Wichtigkeit der Prophylaxe hervorzuheben ist der Name Nosocomial Parotit vorgeschlagen worden

REFERENCES

Arnulf G 1951 Considérations sur les parotidites post operatoires *Mem Acad Chir 77* 544

Faye C & Deffez J P 1969 Les parotidites uremiques *La Vie Medical 50* 2397

Hemenway W G & English G M 1971 Surgical treatment of acute bacterial parotitis *Postgrad Med 50* 114

Korting G W & Kleinschmidt W 1953 *Dermat Wochenschr 128* 772

Laburthe Tolra Y 1969 Les parotidites post-operatoires *La Vie Medicale 50* 2401

Perzik S L 1962 *Surgical management of* acute parotitis *Arch Surg 86* 247

Rausch S 1959 *Die Speicheldrusen des Menschen* p 344–345 Georg Thieme Verlag Stuttgart

Wainwright W W & Becks H 1943 *J Dent Res 22* 403

THE VESTIBU... ...ERE'S DISEASE

A. Øigaa... ...and S. Dorph

From the Department of L... R... ENT Department Rigshospitalet ... Denmark

Abstract The vestibular aquaeduct was evaluated blind in tomograms from patients with Meniere's disease, chronic otitis and from patients without ear disorders. The descending part of the aquaeduct was clearly visible in 95% of the patients without ear disease. In the two other groups it was visible only in about 50%. It is suggested that a narrow or invisible vestibular aquaeduct is an unspecific radiological sign in a variety of ear diseases.

Tomograms of the temporal bone from patients with Meniere's disease or chronic otitis and from patients without ear diseases were studied in a blind analysis in order to assess the diagnostic value of a narrow or invisible vestibular canal in Meniere's disease.

MATERIAL AND METHODS

Group 1 consists of 47 patients with Meniere's disease. All patients fulfilled the following five subjective criteria: (1) hearing impairment, (2) attacks of tinnitus, (3) vertigo, (4) nausea and (5) vomiting. Objectively, all patients had perceptive hearing loss and recruitment measured by the method of Fowler and/or Metz. The disease had lasted for 1 to 20 years (average 6.2 years). In 6 cases the disease was bilateral, giving a total of 53 affected ears. In 38 of the 41 patients with unilateral disease, tomography of the contralateral ear was performed.

Group 2 consists of 35 patients (43 ears) with chronic otitis. Radiologically all ears examined in this group presented blurring of the middle-ear cavity and a variable degree of ossicular destruction.

Group 3. 30 patients referred to the ENT Department with a variety of non-otogenic diseases. These patients volunteered for bilateral tomography.

The age and sex distribution of the patients in all three groups appears from Table I.

A Philips Massiot Polytome was used for the examinations. Tomography was performed in the lateral or a slightly modified lateral projection as originally proposed by Clemis & Valvassori (1968). Sections 1 mm apart, with at least three sections medial and lateral to the common crus of the superior and posterior semicircular canals.

The 194 tomograms were analysed blind in a random order. In each case the vestibular aquaeduct was classified "normal", "narrow", or "invisible", referring to the descending part of the canal.

Table I *Age and sex distribution of patients*
S D =standards deviation

	Sex	Age (years) Average	Range	S D
Meniere s disease	♀ 24 ♂ 23	51 3	26–71	11 8
Chronic otitis	♀ 23 ♂ 12	44 3	10–70	17 5
Normal ears	♀ 20 ♂ 10	44 2	20–75	17 4

aquaeduct was narrow or invisible in 22 out of 43 ears (51%) In the normal group of patients only three out of 60 ears (5%) were "patho logical". The findings in this last mentioned group differ significantly from those in the other groups, including the normal, contralateral ears in patients with Meniere's disease The small differences in the results between ears with Meniere's disease, contralateral ears in Meniere patients and ears with chronic otitis were not statistically significant (Fischer's exact test)

No relationship between the occurrence of pathological tomograms and the age of the patients and the duration of the ear disease could be demonstrated in the series

DISCUSSION

The distinction between an invisible, a narrow, and a normal canal was based on the subjective evaluation of three independent observers Absolute measurements were abandoned because of the uneven diameter of the canal Only the descending part of the canal was considered, since the horizontal (isthmic) part is too narrow to be visualized regularly

The finding of a "normal' aquaeduct in 95% of the ears of patients without ear diseases is in accordance with previous reports (Stahle & Wilbrand, 1974) Our pathological findings also agree with previously published series However, this seems to be the first investiga tion including also a group of patients with other disorders

Since a narrow or invisible vestibular canal was observed with the same frequency in ears with chronic otitis as in those with Meniere s disease, this radiological entity must be con sidered an unspecific diagnostic sign in a variety of ear diseases Furthermore, no con clusive evidence exists in support of the assumption that a tomographically narrow or invisible aquaeduct is actually obliterated (Yuen & Schuknecht, 1972)

ZUSAMMENFASSUNG

Fruhere Mitteilungen haben besagt daß in Ohrtomogrammen von Patienten mit der Meniereschen Krank heit aquaductus vestibuli meistens eng erscheint oder sogar unsichtbar ist Die Bedeutung dieses Befundes in der Diagnose der Meniereschen Krankheit wurde noch nicht festgestellt Die Verfasser machten eine blinde Untersuchtung von Patienten mit der Meniereschen Krankheit von Patienten mit chronischer Otitis sowie von Normalen ohne Ohrleiden Der Aquadukt konnte bei normalen Patienten in 95% demonstriert werden bei Pa tienten mit der Meniereschen Krankheit konnte er am kranken Ohr in 57% und am gesunden kontralateralen Ohr in 53 gezeigt werden Bei Patienten mit chronischer Otitis war er in 51% der Falle nicht zu sehen Der radio-

Table II *Tomographic evaluation of the vestibular aquaeduct in the various groups (N=number of ears)*

Vestibular aquaeduct	Meniere Diseased ears N	%	Menière Contralateral normal ears N	%	Chronic otitis N	%	No ear disease N	%
Normal	23	43 4	18	47 4	21	48 8	57	95 0
Narrow	5	9 4	7	18 4	4	9 3	1	1 7
Invisible	25	47 2	13	34 2	18	41 9	2	3 3
Total (ears)	53		38		43		60	

logische Befund eines engen oder nicht sichtbaren Aquaduktes ist deshalb als unspezifisch anzusehen und ist ohne diagnostischen Wert

REFERENCES

Clemis, J D & Valvassori G E 1968 Recent radiographic and clinical observations on the vestibular aquaeduct (A preliminary report) *Otolaryngol Clin N Am* 339

Stahle, J & Wilbrand H F 1974 The vestibular aquaeduct in patients with Meniere s disease A tomographic and clinical investigation *Acta Otolaryngol* (Stockh) *78*, 36

Yuen, S S & Schuknecht H F 1972 Vestibular aquaeduct and endolymphatic duct in Meniere's disease *Arch Otolaryngol* 96 553

Acta Otolaryngol 82. 282–285, 1976

LATE RESULTS IN TYMPANOPLASTY

Staging the Operation

M Tos

From the ENT University Clinic, Gentofte Hospital Copenhagen Denmark

Abstract Based on the late results of tympanoplasty on 300 ears and of one stage mastoidectomy and tympanoplasty on 296 discharging ears, the problems involved in staging tympanoplasty are discussed The results of one stage operations were found to be satisfactory 2–10 years after operation The frequency of recurrent cholesteatoma was low 3 4% Instead of two-stage operations, re-operation is recommended in cases of functional failure and in cases in which further improvement of hearing can reasonably be expected

Several publications have appeared in recent years recommending planned two-stage operation on ears with extensive pathological involvement of the middle-ear mucosa, destroyed ossicles and cholesteatoma (Austin, 1969, Smith 1970, Sheehy & Crabtree, 1973) Staging was used already in the first few years of the evolution of tympanoplasty, Rambo (1961), in the first stage performed modified radical mastoidectomy using paraffin as a mold to re-form the middle ear and, in the second stage, fenestration Tabb (1963) advocated a three-stage procedure, the first to eliminate inflammation and cholesteatoma, the second to reconstruct the drum, and the third to reconstruct the ossicular chain After the introduction of Silastic, large pieces of the Silastic sheeting were usually removed in the second stage, in which also the ossicles were reconstructed

We have in all cases—even in those of the most severely diseased middle ears—performed mastoidectomy and tympanoplasty in one sitting Based on the late results of this procedure we wish to discuss the problems involved in the staging of operations Most ENT departments in Scandinavia have a limited capacity with regard to operating rooms and beds which oto-surgery has to share with the other sections of the specialty, including broncho-oesophagology and maxillo-facial traumatology Long waiting lists force the otologist to operate on as many patients as possible, and to reduce the number of re-operations to a minimum Of primary importance, however, is the consideration for the patient to whom two operations will mean added mental and physical strain It is, therefore, of value to establish to what extent the results of two-stage operations are better than those of one-stage operations

MATERIAL AND RESULTS

The material is composed of two groups (1) 300 ears which were dry at the time of operation The pathological changes varied from the slightest with central perforations with or without defective ossicles to the severest with adhesive otitis, tympanosclerosis, cholesteatoma in the tympanic cavity and cavities from previous radical operations with absence of ossicles According to the criteria set up by Sheehy & Crabtree (1973), two-stage

operation would have been indicated in 30 of these ears We performed tympanoplasty and in a third also inspection of the antrum or attic (2) 296 ears which were discharging at the time of operation and which did not become dry despite pre operative conservative therapy Sixty three per cent had cholesteatoma in the tympanic cavity and antrum, and the remaining 37% had granulating otitis, or had previously undergone radical surgery elsewhere In over 75% of the cases, the operation could have been performed in two stages, either because of extensive cholesteatoma, pathological mucosa or destroyed ossicles In 43%, the stapes arcade was absent We performed modified radical mastoid ectomy with removal of the posterior osseous wall of the meatus, in 50% leaving a small edge of the bridge In the same sitting, the middle ear and the meatus were reconstructed with fascia, and the cavity obliterated by means of a muscle flap

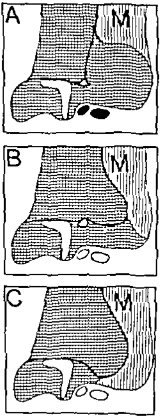


Fig 1 State after conservative radical operation with obliteration of the cavity and reconstruction of the auditory meatus *A* re pneumatized epitympanum and antrum *B* the epitympanum re-pneumatized the rest obliterated *C* the epitympanum is a small open cavity the rest has been obliterated

Hearing

Detailed reports on the hearing results have previously been published (Tos, 1974), and the primary results—3–9 months after the operation—have been compared with the late results—2–10 years after the operation In 4–10% of the cases the late results were found to be poorer than the primary results The most frequent causes of later reduced hearing were adhesive changes in the middle ear and presbyacusia Related to the primary results the late results were not poorer in the group of discharging than in that of dry ears This means that in the long run a successful one-stage reconstruction of a discharging ear can be maintained just as well as that of a dry ear

Discharge and recurrent perforation

In the group of discharging ears 90% were dry during the period of observation 2% continued to discharge while 8% discharged occasionally due to recurrent perforation Among the dry ears only one discharged occasionally all the other remained dry Recurrent perforation of the drum occurred in 8%

Recurrent cholesteatoma

This occurred in 3 4% of the discharging but in none of the dry ears The finding of recurrent cholesteatoma did not lead to difficulties in the tympanic cavity In 40% the epitympanum and antrum were re-pneuma tized (Fig 1*A*) The meatus was completely restored without cavity but its superior wall was soft and consisted of skin and fascia which bulged by the Valsalva test At follow-up

recurrent cholesteatoma, if any, would easily be noticed through this soft wall Likewise, it would be easily recognizable in the 32% of the cases in which only the epitympanum had been re-pneumatized (Fig 1*B*) and the antrum obliterated, and in the 27% in which the epitympanum was an open cavity (Fig 1*C*)

Table I *Hearing results in staged tympanoplasty (Sheehy & Crabtree) compared with those obtained in one-stage tympanoplasty*

	Postoperative bone air gap dB				
	No of cases	*0–10* (%)	*0–15* (%)	*0–20* (%)	*0–30* (%)
Sheehy & Crabtree	273	37		67	85
Tos discharging ears	296		41		73
Tos, dry ears	300		68		91

DISCUSSION

The hearing results obtained in Sheehy & Crabtree's series (1973) are very fine (Table I), especially in view of the extent of pathological changes and ossicular destruction It should be borne in mind, however, that there were twice as many operations and that they were performed by surgeons of wide experience and high technical skill The time of observation was short 6 months in 32%, 1–2 years in 32% and 2–6 years in 36%, and hearing will probably be somewhat reduced at a later time, particularly in the patients with tubal malfunction

Our results of operation on discharging ears in one sitting are somewhat poorer but, with a period of observation of 2–10 years, not so much poorer that we would consider it justified, because of the hearing gain to alter our present practice and employ planned two-stage operations Cases of complete functional failure and cases in which re operation can reasonably be expected to improve the hearing can always be subjected to a second operation at a later time

Another argument against two-stage procedures is tubal function Even though in many cases, poor pre-operative tubal function will improve after the first operation (Tos, 1974), there will always be some ears in which tubal function remains poor after the operation and in which the second operation will not give the desired improved hearing and in which adhesive changes will appear at a later time all the same To prevent such changes Silastic has been used to an increasing extent, and this has been used as an argument for planned two-stage procedures in which the Silastic was removed in the second stage Instead of Silastic, we have lately changed over to a gel film (Falbe-Hansen & Tos, 1975) which can be resorbed and with which we obtain better results than with Silastic The most weighty argument in favour of staging is the high frequency of recurrent cholesteatoma which Sheehy & Crabtree experienced in their series in the tympanic cavity in 25% of 314 revised cases, and in the epitympanum in 35% of 99 re-explorations of the mastoid A high cholesteatoma frequency was found to follow especially the intact wall technique, which was therefore staged to an increasing degree In our series, we found recurrent cholesteatoma in 3 4%, and we do not feel that the fear of recurrent cholesteatoma should indicate staging Our method of operation has been essentially different, however, as we have changed the posterior osseous meatal wall into a narrow bridge and, at the least risk of incomplete removal of the cholesteatoma removed the bridge either entirely or partially The high frequency of recurring cholesteatoma is alarming, and one cannot be certain that the cholesteatoma is definitively removed at the second stage

ZUSAMMENFASSUNG

Auf Grundlage der Spätergebnisse in 300 Ohren in denen Tympanoplastik und in 296 fließenden Ohren in denen Mastoidektomie und Tympanoplastik auf demselben

Sitz vorgenommen wurden werden die mit Stufung der Tympanoplastik verbundenen Probleme diskutiert Die Resultate 2 10 Jahre nach der einstufigen Operation wurden zufriedenstellend befunden insbesondere die Cholesteatom Rezidivfrequenz war niedrig 3 4% Anstelle zweistufiger Operation wird befürwortet die in funktioneller Hinsicht total mißlungenen Falle sowie die Fälle von denen es mit einiger Sicherheit zu erwarten ist daß Neuoperation das Horvermogen noch weiter verbessern wird neuzuoperieren

REFERENCES

Austin D F 1969 Types and indications of staging *Arch Otolaryngol 89* 235

Falbe-Hansen J & Tos M 1975 Silastic or gelatin film sheeting in tympanoplasty *Ann Otol Rhinol Laryngol 84* 315

Rambo J H T 1961 The use of paraffin to create a middle ear space in musculoplasty *Laryngoscope 71* 612

Sheehy J L & Crabtree J A 1973 Tympanoplasty Staging the operation *Laryngoscope 83* 1594

Smith G D L 1970 Staged tympanoplasty *J Laryngol 84* 757

Tabb H G 1963 The surgical management of chronic ear disease with special reference to stages surgery *Laryngoscope 73* 363

Tos M 1974 Tubal function and tympanoplasty *J Laryngol 88* 1113

— 1974 Late results in tympanoplasty *Arch Otolaryngol 100* 30?

Acta Otolaryng 82 286–288, 1976

SECRETORY OTITIS MEDIA

Pathology and Pathogenesis Related to Clinical Picture

M Tos

From the ENT University Clinic Gentofte Hospital Copenhagen Denmark

Abstract Secretory otitis is divided into three stages and from a description of the pathogenic processes taking place in each stage it is attempted to explain how secretion is generated and why it stops The initial stage is characterized by inflammatory changes by way of capillary dilatation and proliferation lymphatic infiltration increased goblet cell density and formation of mucous glands the secretion is exudative with admixture of mucus The secretory stage is dominated by secretion of mucus from the metaplastic mucosa with extremely high density of goblet cells and glands In the degenerative stage the production of mucus abates because the *goblet-cell density decreases and the glands degenerate*

The fluid accumulating behind the intact drum in chronic secretory otitis is a product of various processes in the tubal and middle ear mucosa These processes have started long before the principal symptom—hearing loss—manifests itself, but also after the clinical symptoms have abated processes are taking place in the middle ear and the auditory tube which may lead either to cure or relapse of the disease In the present study, these are reviewed and related to the clin[illegible] ire of the disease

For a century, many inves[illegible] concerned with [illegible] on of h[illegible] middle ear secret[illegible] ture, see Tos, [illegible] era[illegible] been advanced [illegible] er[illegible] (Politzer, 1878), [illegible] on tion that the [illegible] from the low p[illegible] *exudation (I*[illegible] assumes that the fluid is an exudate with a higher protein content than serum The *demonstration of the secretory ability of the* middle ear mucosa (Sade, 1966 Bak Pedersen & Tos 1971) marks a great step forward and forms the basis of the *secretory theory*

MATERIALS AND METHODS

The entire tubal and middle ear mucosa was studied in necropsy specimens from foetuses prematures and infants normal children children with secretory otitis in the initial stage normal and abnormal adults Furthermore a study was made of over 5000 biopsy specimens taken from the middle ear mucosa during surgery in 496 patients with disorders of the middle ear 85 were children with secre*tory otitis in the secretory stage* and 48 mainly adults had chronic adhesive otitis which is the terminal stage of secretory otitis

All specimens were stained by the PAS-alcian blue whole mount method and the density of [illegible] cells and mucous glands was determi[illegible] atively Subsequently the [illegible] cut into serial sections stained [illegible] lin eosin and alcian blue and [illegible] ed

[illegible]

The [illegible] stages *(I)* [illegible] from the action

of the aetiological factor on the middle-ear mucosa till secretion accumulates and causes symptoms, during which the first metaplastic changes occur in the middle ear mucosa, clinical symptoms are mild, originating mainly from the tubal occlusion which is always present. (2) The *secretory stage*, which is dominated by mucous secretion with the well-known symptoms of fluid accumulation. (3) The *degenerative stage* during which the mucus production decreases and the clinical symptoms vanish, provided that irreversible adhesive changes have not developed—if so, the disease has gone into its terminal stage, chronic adhesive otitis.

PATHOLOGY AND PATHOGENESIS

The *initial stage* is characterized by inflammatory changes starting in the lamina propria by way of capillary proliferation and dilatation as well as lymphocytic infiltration. These changes soon induce hyperplasia and differentiation of the epithelial cells with subsequent metaplasia of the epithelium into pseudostratified columnar ciliated epithelium with increased goblet cell density and formation of mucous glands (Tos & Bak-Pedersen, 1975*a*). The most crucial process is the gland formation which sets in with invasion of undifferentiated epithelial cells into the lamina propria: these cells differentiate into ciliary and mucous cells, the glands become canalized and secretion and transport of mucus from the glands to the epithelial surface commence. The final result of the metaplastic changes is a middle-ear mucosa reminiscent of that of the respiratory tract, i.e. with great density of goblet cells and glands with a high mucus producing capacity. It is obvious that inflammatory and metaplastic changes of this type cause exudation through the epithelium, an exudation which is accompanied by expulsion of epithelial cells and lymphocytic migration. Owing to the considerably enhanced number of goblet cells, the production of mucus is also increased, and the exudate therefore becomes admixed with mucus.

The *secretory stage* is dominated by secretion of mucus from the metaplastic middle ear mucosa. The number of goblet cells (Tos & Bak Pedersen, 1972, 1975*b*) and active mucous glands is greatly increased. The inflammatory processes continue, in the severest cases leaving the mucosa thickened and polypous. Exudation also occurs at this stage, but, since the mucus-producing capacity of the mucosa is now maximal, the secretion is predominantly mucous. Quantitative studies of goblet cells and mucous glands revealed great individual differences, which is in good agreement with the varying degrees of severity of the disease. With low density of mucous glands the accumulation of mucus will be small, and the clinical symptoms will abate rapidly without or after a short treatment. Conversely, with pronounced metaplasia and a high density of mucous elements, mucus will continue to accumulate, and repeated drainage is required.

It is a well known fact that even in the severest cases the accumulation of mucus will gradually diminish, and this is possible only if the mucus producing capacity of the mucosa is impaired. Processes to this effect take place in the *degenerative stage*. The number of goblet cells decreases, and the inflammatory changes subside, the epithelium returns to normal cubical epithelium in one or two layers, or is transformed by metaplasia into secretorily inactive stratified squamous epithelium (Tos, 1976). The most important process is the degeneration from active to completely inactive cystic glands. This degeneration has been demonstrated quantitatively: in the secretory stage 93% of the glands were found to be active against only 11% in the terminal stage (Tos & Bak Pedersen, 1973). The end result is a decline in both exudation and mucus production. Provided that tubal function returns to normal, the remaining secretion will be carried to the

rhinopharynx, and the patient becomes free of symptoms Whether secretion will reaccumulate in the middle ear depends on the mucus producing capacity of the mucosa and on the ability of the tube to carry the mucus away

DISCUSSION

Based on the description of the secretory pathogenesis and of the pathological findings in the mucosa, an attempt is made to explain how middle-ear secretion is generated, and why it ceases The secretion must be regarded as a mixture of exudate and mucus, with the exudate dominating in the initial and the mucus in the secretory stage At all stages fluid is also absorbed (Lim, 1975)

Whether transudation *ex vacuo* also occurs in the initial stage could not be proved histologically, but we could prove that exudation takes place which, at all stages, accounts for the non mucous element of the secretion In the initial and the secretory stage, the middle-ear pressure is always negative During the period with the drain operating well, the function of the auditory tube is usually markedly pathological (Renvall, 1975) In the egenerative stage i e after expulsion of the rain, the middle ear pressure is likewise egative Tubal occlusion may be present for a long time without giving rise to transudation *ex vacuo* By experimental tubal occlusion with simultaneous drainage of the drum no fluid formed in the middle ear (Proud & Odoi, 1970), and this is taken to be the most important evidence in support of the hydrops *ex vacuo theory In my view, these experiments* demonstrate only that tubal occlusion is the principal aetiological factor in the production of fluid, whereas they reveal nothing of how the fluid is generated Our histological studies of the infantile middle-ear mucosa however showed the same changes in tubal occlusion as those seen in the initial stage of secretory otitis

ZUSAMMENFASSUNG

Sekretorische Otitis wird in drei Stadien eingeteilt und *durch eine Beschreibung der pathogenetischen Prozesse* jedes Stadiums wird versucht zu erklaren wie die Sekretion entsteht und warum sie aufhort Im Initialstadium werden inflammatorische Veranderungen in Form von Kapillardilatation und Proliferation Lymphozyteninfiltration Vermehrung der Becherzellanzahl sowie Glandelbildung gesehen das Sekret ist ein mit Schleim *vermischtes Exsudat Im Sekretionsstadium* dominiert die Sekretion von Mukus aus der metaplastisch veranderten Schleimhaut mit sehr hoher Becherzell und Glandeldichte Im Degenerationsstadium nimmt die Mukusproduktion ab weil die Becherzelldichte abnimmt und die Drusen degenerieren

REFERENCES

Bak Pedersen K & Tos M 1971 The mucous glands in chronic secretory otitis media *Acta Otolaryngol* (Stockh) *72* 14

Lim D J 1974 Functional morphology of the lining membrane of the middle ear and Eustachian tube *Ann Otol Rhinol Laryngol* Suppl *11* 3

Politzer A 1878 *Lehrbuch der Ohrenheilkunde* 1–2 p 84 Enke Stuttgart

Proud G O & Odoi H 1970 Effects of Eustachian tube ligation *Ann Otol Rhinol Laryngol 79* 30

Renvall U 1975 *Secretory otitis media studies by Eustachian tube function test and impedance audometry* Elanders Tryckeri Goteborg

Sade J 1966 Pathology and pathogenesis of serous otitis media *Arch Otolaryngol 84* 297

Tos M 1976 Pathologie und Pathogenese der Chronischen Sekretorischen Otitis im Kindesalter *HNO 24* 37

Tos M & Bak Pedersen K 1972 Density of mucous glands in a biopsy material of chronic secretory otitis media *Acta Otolaryngol* (Stockh) *75* 55

— 1973 Density of mucous glands in chronic adhaesive otitis media *Arch Ohren Nasen Kehlkopfheilkd 206* 39

— 1975*a* The outset of chronic secretory otitis media A histopathological study of the earliest stage *Arch Otolaryngol 101* 123

— 1975*b* Density of goblet cells in chronic secretory otitis media Findings in a biopsy material *Laryngoscope 85* 377

Zöllner F 1936 Widerstandsmessungen an der Ohrtrompete zur Prufung ihrer Wegsamkeit Ein neues Verfahren und bisherige Ergebnisse an Ohrgesunden und kranken *Arch Ohren Nasen Kehlkopfheilkd 140* 137

Acta Otolaryngol 82 289–293, 1976

THE NATURAL COURSE OF MENIERE'S DISEASE

R Haye and Sv Quist Hanssen

From the Department of Otolaryngology University Hospital Rikshospitalet Oslo Norway

Abstract The case history and disability of 111 patients suffering from Meniere s disease are reported Observed for more than 5 years the patients have had no or only medical treatment in most cases of short duration The number of patients disabled and their degree of disability seems to justify surgical treatment in patients with frequent and incapacitating attacks of vertigo

In 1861, Prospere Meniere described the pathological picture of the disease now bearing his name, and claimed that its cause had to be found in the labyrinth (Opheim, 1965) 77 years later, Hallpike & Cairns (1938) demonstrated the "endolymphatic hydrops" in the labyrinth of two patients with Meniere's disease who died after section of the acoustic nerve The endolymphatic hydrops has been described by a number of investigators (Altman et al , 1943, Lindsay et al 1958, Golding Wood, 1960B, Seymour, 1960, Kristensen, 1961, Kohut et al , 1972)

Even if the distension of the cochlear duct and the saccule and ruptures of the endolymphatic system (Schuknecht et al , 1966, Kohut et al , 1972) should be the cause of the symptoms, we do not know how the distension arises (Lindsay 1947, Egmond et al , 1956, Williams, 1965) nor how it produces the symptoms of Meniere s disease

The lack of knowledge has laid the field open to hypotheses based on
belief A number of causes l
negative pressure in the midd

1966, Lall, 1969), salt and fluid dysregulation (Schick, 1943, Hoogland, 1952, Tokita et al 1971, Angelborg et al , 1973, Thomsen et al , 1974) (denied by Golding-Wood, 1960*a*), metabolic derangement (Shambaugh Jr, 1959, Powers 1972, Pulec, 1972, Godlowski, 1972), allergy (Wilson, 1972, Pulec et al , 1973), vasomotor dysfunction (Mygind, 1947, Hoogland, 1952, Seymour et al , 1953, 1960, Derlacki, 1956, Wildhagen, 1958, Golding Wood, 1960*c*, Williams, 1965) Some authors stress the importance of psychic factors (Fowler et al , 1956, Siirala et al , 1965, Williamson et al 1971), others find them of no importance (Pulec, 1972) and think that the psychic reaction is a result of the disease (Jongkees, 1964) Suggested causes of Meniere's disease are numerous, and so are also the treatments proposed (Sjoberg, 1964, Hinchcliffe, 1972)

According to some authors the majority of patients are cured or sufficiently improved by medical treatment which is dominated by vasodilators, histamines, diuretics and osmotically active substances (James et al , 1960, M Portmann, 1964, G Portmann, 1965, House 1965) Others find that drugs have no effect and think the patients are cured without any treatment (Permin et al 1957, Jongkees, 1964 House 1965 Pickard 1967) In spite of this, surgical treatment has been widely used

Table I *Age distribution*

Years	≤30	31–40	41–50	51–60	61–70	71–80	≥81	Total
No of patients	3	5	14	39	27	19	4	111

Table II *Occupation*

	Men	Women
Farm	10	
Office	23	2
Industry	28	1
Teaching	6	
House wife		29
Other	12	

obtained good results by grommet insertion into the ear drum Arslan's (1969, 1972) hyperosmosis treatment by sodium chloride crystals on the round window membrane is supported by the experimental investigations of Galle and Siegels (1975) Cervical sympathetic block or sympathectomy (Hoogland, 1952, Seymour, 1953, 1960, Golding-Wood, 1960*c*), section of the eighth nerve (Altmann et al , 1943, Golding-Wood, 1960*b*), or only its vestibular part (Fluur et al , 1965, Malmros et al , 1966, Stupp et al , 1974) have given good results, but also *serious complications* (Hallpike et al , 1938, Altmann et al , 1943) At the present time, operations on the labyrinth are most frequently used, as "drainage procedures" (M Portmann, 1964, Cawthorne 1964, G Portmann, 1965, Fick, 1965, House 1965, Turner et al , 1973), destruction of the labyrinth by ultrasound (Brain et al , 1960, James et al , 1960, Sjøberg, 1963, Pennington et al , 1970, Oberhand, 1973, Stahle et al , 1973) or labyrinthectomy (Golding Wood 1960*b*, Cawthorne et al , 1967) Even these operations have not been without complications

Meniere's disease attacks both ears with a frequency ranging from 10 to 70 per cent (Altmann, 1943, Golding-Wood, 1960*c*, Cawthorne, 1964, Opheim, 1965, Bergman et al , 1966, Cawthorne et al , 1967, Rudert et al 1974) The risk of bilateral disease has made some authors refrain from surgical treatment whereas others have opened the endo-

Table III *Observation time from first attack of vertigo*

Years	5–9	10–14	15–19	20–24	25–29	30–34	35–39	≥40
No of patients	24	27	29	18	4	3	4	2

Table IV *Age at first attack of vertigo*

Years	≤10	11–20	21–30	31–40	41–50	51–60	61–70	>70
No of patients	2	10	24	30	26	15	3	1

Table V *Symptoms at first and later attacks of vertigo*

Symptom	Full ness in the ear	Tinnitus	Hearing loss	Vertigo	Nausea	Vomit	No in form	No of pats
First attack	53	83	79	111	102	93		111
Later attacks	51	97	101	106	87	68	1	107

Table VI *Duration of first attack of vertigo and longest duration of later attack of vertigo (number of patients affected)*
H=hours D days

Duration	<½ H	½–5 H	6–24 H	1–7 D	≥7 D	No inform	Total
First attack	14	39	30	19	4	5	111
Later attacks	14	32	27	19	6	9	107

Table VII *Number of attacks of vertigo a year in bad periods*

No of attacks	0	1–3	4–6	7–29	≥30	No inform
No of patients	4	21	18	17	35	16

lymphatic sac in both ears with good results (Rudert et al , 1974)

Surgical treatment may be justified, provided it can, on long terms offer the patient a situation decisively preferable to that given by the natural course of the disease In order to know this course we have, therefore studied a group of practically untreated patients with Meniere's disease

The diagnoses were based on a characteristic case history, audiological findings and in almost all patients on additional results of vestibular, otological, ophthalmological neurological and X ray examinations and routine laboratory tests (Opheim et al 1957) A questionnaire was sent to 138 patients examined in our department from 1960 to 1970 111 replied to it Four had been treated surgically and their case histories are included only until the time of operation 31 patients had received no treatment at all, 70 had medical treatment for a few weeks, four also massage on the neck, and two repeated inflation of the Eustachian tube Only 10 patients used nicotinic acid for any length of time, but continued to have symptoms

As appears from Table I 72% of the patients who answered the questionnaire were between 41 and 70 years, 2/3 men, 1/3 women (Table II) The inverted relation was found by Permin et al (1957)

Our department has received all patients referred for vertigo and hearing loss without regard to domicile age, sex and occupation (Table II) Vertigo is the alarming symptom and we have therefore as Siirala et al (1965) regarded the first attack of vertigo as the onset of the disease The observation period (Table

Table VIII *Longest period without attack of vertigo*
Four patients operated upon are not included there is no information on 14
M=months Y=years

	≤1 M	1–6 M	7–12 M	2 Y	3 Y	4 Y	5 Y	6 Y	7–9 Y	10–13 Y	≥14 Y
No of patients	11	6	18	7	9	6	6	5	13	9	3

Table IX *Both ears affected*

	Time interval betwee[n]		
Years	0	1–5	(
No of patients	13	11	;

Table X *Hearing*

	No of patients
Good	21
Medium	40
Poor	43
No inform	7

III) is 5 years or more for all the patients, for 60 more than 15 and for 31 more than 20 years The age at the first attack of vertigo is shown in Table IV As found by others (Permin et al , 1957, Golding-Wood, 1960*b*), 40% of the patients had hearing symptoms preceding the first attack of vertigo, in 2/3 by less than 3 years, in five patients by more than 10 The first attack was usually dominated by vestibular symptoms, later attacks more by tinnitus and hearing loss (Table V) The duration of the first and the longest later attack were almost the same (Table VI) and in about one-half of the patients less than 6 hours In bad periods, 1/3 had more than 30 attacks of vertigo per year (Table VII) The longest period without attack of vertigo was in 39% of the patients 5 years or more (Table VIII) Four patients had only one attack of vertigo in addition to the hearing symptoms

As previously mentioned, 4 patients had been treated surgically for frequent and incapacitating attacks of vertigo Two had been free from vertigo for 5 and 9 years, respectively, after ultrasound treatment, one for 6 months after labyrinthectomy one for 16 years after section of the eighth nerve before he had new attacks and impaired hearing in the other ear One patient was treated with salt crystals in the middle ear with no effect Five additional patients had been free from vertigo, 2 for 10 years, one of them after drinking 2½ litres of milk a day for some time, the other after a cure with herb tea, 3 after massage of the neck, one of them for 14 years As many others, two of the latter had a slight osteochondrosis of the cervical spine

In 41 patients (37%) both ears were affected, in 24 at intervals of less than 6 years (Table IX) 51 patients had a driver's licence 6 had a case of Meniere's disease among their relatives 83 patients were hard of hearing (Table X) Eight had tried a hearing aid without success, 30 used an aid, but only 24 benefited from it Other complaints and disabilities are given in Table XI Our study confirms the experience of others, viz that Meniere's disease shows a highly capricious and varying course which cannot be predicted in the individual case This and the long remissions prevent a realistic evaluation of the results of treatment

After medical treatment, or no therapy at all, the majority of patients with Meniere's disease get rid of their attacks of vertigo, but in most cases only after several years Sequelae of the disease, tinnitus, hearing loss, imbalance and fear of new attacks of vertigo are frequently incapacitating (Table XI)

Thanks to developments in surgery on the labyrinth during recent years we therefore think it reasonable to try this kind of treatment in cases of Meniere's disease with frequent and incapacitating attacks of vertigo It should not be forgotten, however, that each attack may be the last one Whatever the treatment chosen our series confirms the value of psychotherapy (Sjøberg, 1964) and the view expressed by Jongkees (1964), viz to gain the patient's confidence is the basis for all treatment of Meniere s disease

Table XI *Complaints and disabilities (n=111)*

	Present	No inform
Nervousness	61	7
Difficult walking at dusk	51	
Accidents from vertigo	12	2
Unfit for work		
temporary first year	20	
lasting	25	
Difficulties		
at work	57	6
at home	32	6
among friends	36	6
Pensioned before time	35	7

ZUSAMMENFASSUNG

Die Krankengeschichte und die Invalidität von 111 Patienten mit der Meniereschen Krankheit werden mitgeteilt Beobachtet mehr als fünf Jahre hatten die Kranken keine Behandlung bekommen oder nur eine kurzdauernde medikamentelle Die Zahl der Kranken die stark behindert waren und der Grad ihrer Invalidität scheint chirurgische Eingriffe zu berechtigen in jenen Kranken die von haufigen und invalidizierenden Schwindelanfällen belastigt sind

List of references may be requested from the authors

Acta Otolaryngol 82 294–296, 1976

LITHIUM TREATMENT OF MENIERE'S DISEASE

Results of a Double blind Cross over Trial

J Thomsen, P Bech, A Geisler, S Prytz, O J Rafaelsen,
P Vendsborg and K Zilstorff

*From the University ENT Department and Psychochemistry Institute
Rigshospitalet Copenhagen Denmark*

Abstract 21 patients with Meniere s disease participated in a double blind cross over trial with 6 months lithium treatment and 6 months placebo treatment Even if the results were not completely unequivocal no difference was found between lithium and placebo treatment The results might imply that both other medical as well as surgical treatment of Meniere s disease exert a pure placebo effect so far no *controlled* investigation has been published establishing the value of surgical treatment of Meniere s disease

On the assumption that the hydrops of the endolymphatic space present in Meniere's disease is due to a defective transport of water and ions between the endo- and perilymphatic space, the authors in an open pilot study treated patients with this disease with lithium carbonate, as lithium among many biological effects acts on the transport of ions across membranes (Thomsen et al 1974) The results showed that in 70% (21 patients) the *effect of the treatment* was *excellent* as assessed by the reduction in the frequency and severity of the attacks Although a placebo effect could not be ruled out in the open trial the high and persisting effect justified continued investigations into the effect of lithium upon Meniere's disease

We therefore planned two controlled investigations of the possible effect of lithium upon Meniere's disease

I The 21 patients who responded positively in the open trial now participated in a closed double blind cross over trial, with 6 months' treatment and 6 months' placebo treatment It is the results of this trial that is presented here

II Another double blind cross over trial *this time with fresh cases of Meniere s disease* with the same design as mentioned above The results of this investigation will be presented in a subsequent publication

Each week, all the patients filled in a dizziness questionnaire in which the frequency of the attacks, as well as the duration and severity of the individual attack were recorded All symptoms within the Meniere symptom complex (hearing loss, tinnitus, dizziness nausea, vomiting, pressure in the ears) were also recorded

The patients' own statements could be directly transferred to punched cards and the *computer* At the end of the trial, the patients' scores, as well as the total score and the scores for the individual symptoms could be compared for the two treatment periods

The serum-lithium concentration was checked every 2 weeks All the patients were kept on serum lithium levels within the therapeutic range, i e at least for the manio-depressive psychosis, 0 7–1 0 mmol/l If the patients' serum lithium concentration were found to be outside this range, changes in the tablet intake were made, at the same time a

patient in the placebo period had a similar correction of the tablet intake This control and correction of the tablet intake was performed by a physician at the Psychochemistry Institute, who otherwise had nothing to do with the treatment of the patients This paired correction was made to prevent the investigator from gaining an insight into which treatment period the patients were

Each month the investigator filled in a questionnaire as regards any unwanted effect of the lithium treatment Furthermore, he tried to evaluate the patients' present state of disease, giving scores from 0 to 10 depending on the severity of the disease

After the termination of both treatment periods the patients were asked to give their opinion as to which period had been the best, i e the period in which they had had the fewest attacks Audiometric examinations with tone audiogram, speech audiometry, and recruitment tests (Fowler and Metz) was performed at regular intervals

RESULTS

One patient discontinued treatment during the trial, leaving us with 20 patients who completed the trial, each with 6 months' placebo and 6 months' lithium treatment Five preferred lithium, i e , felt best in the active period, while 5 patients preferred the placebo period, 10 patients had no preference

In making up the patients' scores, both the total scores, as well as the scores for the individual symptoms, it turned out that 10 patients had so mild symptoms that their scores did not allow statistical calculations They were by and large free of attacks the entire year The remaining 10 patients obtained scores of a reasonable size permitting statistical calculations No difference could be found between the scores in the lithium period and the placebo period, either in the total scores or in the scores for the individual symptoms There was not even a tendency in the figures

The investigator's monthly evaluation of the patients' disease did not reveal any difference between active and placebo periods, nor did the audiometric tests show any difference

DISCUSSION

An uncontrolled study can be used for a primary investigation of a drug or other method of treatment, but is worthless for a real evaluation of the therapeutic effect This necessitates a controlled trial, which implies that, in addition to the treated group there must be a control group The investigation must be double-blind, i e , neither the investigator, nor the patient must be aware of which group the patient belongs to, or what treatment he is receiving at any given time

There are a number of possible designs for the controlled trial Total randomization of the patients, so that they are randomly assigned to a treatment or control group, is hardly possible for the investigation of Meniere's disease The two groups established by this means will most probably be incomparable, especially if they are small

It is also possible to use randomized paired groups in which the treatment and the control group are comparable with regard to factors like age, sex, duration and severity of the disease It is, however, difficult to use this design in dizzy patients, in this case with Meniere's disease, since it is almost impossible to find completely comparable groups with respect to a number of factors which are difficult to define such as the mental constitution of the patients

The above-mentioned techniques of investigation have some advantage over the cross over technique, since the two groups are observed simultaneously, and spontaneous variations in the course of the disease are of less importance

When completely comparable groups cannot be obtained, the cross-over technique is the most suitable, the patient is treated with the

active drug and placebo for two consecutive periods in random order The advantage of this method is that interindividual variations disappear and the treatment and control groups are as identical as possible, since the patient serves as his own control

The disadvantage of the cross over technique is that spontaneous variations in the course of the disease, which indeed exist in Meniere's disease, will influence the result, because it is necessary to compare two separate periods of time Another disadvantage of the cross over design is that both the active drug and the placebo may continue to act after their withdrawal and thus have a disturbing effect in the subsequent period

In evaluating our patients both of the above mentioned phenomena may have influenced the results Our patients had all had Meniere's disease for a number of years, since they had all prior to this investigation received treatment with lithium carbonate for an average period of 18 months It is therefore possible that the spontaneous course of the disease is to blame for the fact that the 10 patients did not experience any attack at all in the trial period It is however, also possible that this is due to a carry over effect from the previous lithium treatment

Concerning the other 10 patients in whom it was possible to register attacks in the entire trial period, with no difference between the two periods this might also be due to either a spontaneous stabilization of the disease or a carry over effect from the previous treatment

Bearing these reservations in mind we must, however say that the results as they are here show that lithium carbonate has no effect upon the symptoms in Meniere's disease On the other hand, we believe that it is still justified to complete the trial in progress with fresh cases of Meniere's disease The carry over effect will here probably be eliminated, and the patients are treated so early in the course of the disease that it has not yet burned out

If we assume that lithium carbonate has no effect upon Meniere's disease, we are in a situation where we, in an open, uncontrolled trial, obtained an excellent effect in 70% of the patients with an inactive placebo preparation This may imply that the current treatment of patients with Meniere's disease, whether medical or surgical, may be a pure placebo effect This might, at least in the case of the surgical treatment, influence the indication for operation, since up to this date, no *controlled* investigation has ever been published establishing the value of surgical treatment of Meniere's disease

ZUSAMMENFASSUNG

21 Patienten mit der Menіereschen Krankheit wurden in *einem doppelt blinden Versuch kreuzweise* durch 6 Monate mit Lithium und 6 Monate mit Placebo behandelt Obwohl die Ergebnisse nicht absolut eindeutig sind wurde keine Unterschied zwischen Lithium und Placebobehandlung festgestellt Es könnte an den Ergebnissen liegen daß sowohl andere medizinische als auch chirurgische Behandlung der Meniereschen Krankheit nur eine reine Placebowirkung ist indem keine *kontrollierte* Arbeit eine positive Wirkung chirurgischer Eingriffe festgestellt hat

REFERENCES

Thomsen J Bech P Geisler A Jorgensen M Balslev Rafaelsen O J Terkildsen K Udsen J & Zilstorff K 1974 Meniere s disease Preliminary report of lithium treatment *Acta Otolaryngol* (Stockh) *78* 59

Acta Otolaryngol 82 297–300, 1976

ACCUMULATION OF ^{14}C LIDOCAINE IN THE INNER EAR

Preliminary Clinical Experience utilizing Intravenous Lidocaine in the Treatment of Severe Tinnitus

S Englesson, B Larsson, N G Lindquist, L Lyttkens and J Stahle

From the Departments of Otolaryngology Anaesthesiology and Toxicology University of Uppsala Uppsala Sweden

Abstract Clinical experience has demonstrated that intra venously administered local anaesthetics have a mitigat ing effect on severe tinnitus In an attempt to gain some insight into the mechanism of this effect autoradiography of the inner ear of young pigmented rats was performed after intravenous injection of ^{14}C lidocaine Some accu mulation of lidocaine was found in the modiolus but almost none in the stria vascularis A large accumulation was observed in other melanincontaining tissues such as the hair follicles and uvea The *in vitro* accumulation of ^{14}C labelled lidocaine adsorbed on melanin granules was low (12%) as compared with that of other drugs such as kanamycin (89%) and chloroquine (85%) These autoradiographic results indicate that lidocaine has an effect upon the inner ear in addition to its previously demonstrated effect on the CNS

Tinnitus is a great therapeutic problem often affecting patients with Meniere s disease otosclerosis and various conditions of sensory neural deafness The patho physiological basis of this subjective tinnitus is still un known It remains to be explained whether it arises in the cochlea, the spiral ganglion or the afferent pathways on the one hand or originates more centrally through the incorrect coding' of the normal flow of impulses on the other

Local anaesthetic agents administered intra venously can temporarily reduce tinnitus (Bárány, 1935, Fowler 1953) an observation which was confirmed by Gejrot (1963) who used lidocaine in the treatment of vertigo in attacks of Meniere's disease According to Bernhard & Bohm (1954), lidocaine blocks central nervous activity and has therefore been used to control epileptic fits On this basis, the therapeutic effect of intravenously administered lidocaine on tinnitus has been interpreted as a blockage of the CNS

The effects of topical lidocaine hydrochloride upon the inner ear of cats were studied by Rahm et al (1962) They reported that lidocaine without epinephrine had toxic effects upon the cochlea evidenced by a reduction in the magnitude of the electrical response, which persisted for up to three days On the other hand, addition of epinephrine in a concentration of 1 100 000 prevented such effects They therefore advised against the use of topical lidocaine without epinephrine

Ward & Honrubia (1969) studied the effects of dilute concentrations of lidocaine administered as a micro perfusion of the scala tympani on the electrical potentials of the inner ear of guinea pigs They reported reversible decreases in the cochlear microphonics (CM) and eighth nerve action poten tials (AP) The relatively greater sensitivity exhibited by the AP, as compared with that of the receptor potentials, was in agreement with the observation of the effects of local anaesthetics on the behaviour or peripheral nerves and other end-organ receptor potentials

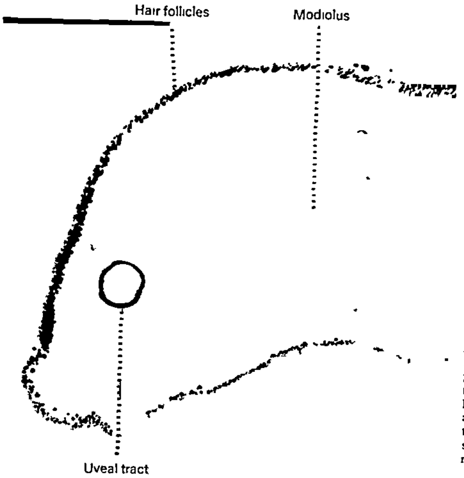


Fig 1 Detail of an autoradiogram of a young pigmented rat 1 hour after an intravenous injection of ^{14}C lidocaine There is a large accumulation in the uveal tract and hair follicles and some accumulation in the modiolus

On the basis of previous findings of the accumulation of drugs on melanin (Potts, 1962 Lindquist 1973, Lindquist & Ullberg, 1975) on the one hand and clinical experience of the mitigating effect of intravenously administered local anaesthetics on severe tinnitus on the other we assumed that the therapeutic effect was due to some local action of lidocaine on the inner ear The experimental and clinical results of our studies are reported below

RESULTS

"Adsorption of ^{14}C-labelled lidocaine by melanin

In vitro investigations, using beef eye melanin according to Potts, showed that ^{14}C labelled lidocaine has a relatively low melanin affinity (12%) This figure should be compared with the uptakes of kanamycin (89%), chloroquine (85%) streptomycin (60%) and salicylic acid (0%) (Lindquist, 1973)

Whole body autoradiography

Previous autoradiographic investigations of chloroquine labelled with ^{14}C have shown an accumulation of the drug on the melanin in the inner ears of young pigmented rats, whereas no accumulation occurred in the inner ear of an albino rat (Dencker & Lindquist, 1975)

Autoradiography at intervals varying between 5 min and 28 days, following the intravenous injection of ^{14}C-labelled lidocaine, has shown accumulation and retention of lidocaine in the melanin bearing tissues of young rats (Fig 1) The most obvious accumulations were found in the uvea and the hair follicles

In the inner ear a sparse accumulation was noticed in the modiolus in pigmented rats up to 24 hours after the injection whereas no accumulation could be seen at longer survival times Unlike chloroquine no accumulation could be detected in the stria vascularis Albino rats showed no accumulation of lidocaine at all in the inner ear

Toxicological studies

The ability of chloroquine and lidocaine to accumulate in melaninbearing tissues prompted us to study patients who had received lidocaine anaesthesia (dosage 6 mg/kg body weight per hour) The total dose of lidocaine per patient was 1000–1500 mg Hearing and sight were checked before and one day after the anaesthesia No functional disturbances related to lidocaine were noted

Therapeutic trials

Lidocaine treatment was given in the form of repeated intravenous injections in an attempt to reach a high serum level and a high uptake of lidocaine in the inner ear The dose was 0 5–2 0 mg/kg body weight given intravenously 10 times daily for 5 days The treatment series comprised 8 patients with severe and disabling tinnitus (Meniere s disease 3 post traumatic inner ear lesion 1 advanced otosclerosis 2 extensive sensory neural deafness 2) Two patients were totally refractory to the treatment All the remaining 6 patients gained freedom from tinnitus lasting from 2–5 minutes after one injection up to several days after completion of the treatment series Two of these 6 patients reported a considerable reduction of their tinnitus lasting two months and then followed by a gradual return to the primary intensity level Repeated courses of treatment were given in the two lastmentioned cases again with ameliorating effects No serious side effects occurred

DISCUSSION

There is an urgent need for some long term treatment to reduce severe subjective tinnitus Since the early thirties intravenously administered local anaesthetics have been known temporarily to reduce tinnitus The local application of anaesthetics in the middle ear has the same ameliorating effect The topical treatment however seems to have ceased owing to the toxic effects noted in experiments on animals The observation of the capacity of melanin to take up various drugs led us to repeat the therapeutic trials with intravenous administration of local anaesthetic agents after having shown a discrete accumulation of radioactively labelled lidocaine in the modiolus of young rats

The *in vitro* accumulation of lidocaine on melanin is much less than that of chloroquine Toxic effects of prolonged treatment with chloroquine have been reported not only in the form of retinopathy but also in the inner ear (Hart & Naunton 1964) Lidocaine on the other hand has not such toxic effects as far as we know In order to shed further light on that question a series of 15 children who in 1969–70 all received lidocaine (4 mg/kg body weight per hour) for several weeks for spasms after birth will be examined with respect to hearing and vision

Autoradiographic studies on young rats have disclosed an accumulation of lidocaine on the melanin in the uvea the skin and to some extent the modiolus Unlike chloroquine however no detectable amounts of lidocaine could be traced in the stria vascularis and in the planum semilunatum Two possible explanations of the topographically different uptakes of these two drugs may be considered First ^{14}C labelled lidocaine in detectable dosages was not taken up by the scanty melanin in the stria vascularis This possibility is supported by our finding of a sparse *in vitro* uptake of lidocaine on melanin granules compared with that of chloroquine Secondly there may be a discrete impediment to lidocaine in relation to the stria vascularis The latter hypothesis may be in accordance with the complex adsorption pattern of melanin An actual example of this is genta

mycin, a drug which is amply taken up by melanin granules *in vitro* but not *in vivo*

Our finding of an accumulation of lidocaine in the modiolus is in good agreement with the results of Ward & Honrubia (1969), who reported that lidocaine has a more pronounced paralysing effect on the eighth nerve action potentials, compared with its effect on the receptor potentials The mitigating effect of lidocaine on tinnitus may thus to some extent be due to a presumed eighth nerve action potential anaesthesia The melanin content of the inner ear, especially in the modiolus which probably varies among individuals, may be of essential significance for the therapeutic results

ZUSAMMENFASSUNG

Klinische Erfahrung hat gezeigt daß intravenos verabreichte Lokalanasthetika einen mildernden Effekt bei schwerem Tinnitus bewirken Bei dem Versuch einen Einblick in den Mechanismus dieses Effektes zu gewinnen wurde eine Autographie der Innenohren von jungen pigmentierten Ratten durchgefuhrt und zwar nach der intravenosen Injektion von C^{14} Lidocain Eine gewisse Anhaufung von Lidocain kam im Modiolus vor aber fast keine in der Stria vascularis Eine große Anhaufung wurde in anderen melaninenthaltenden Geweben beobachtet wie Haarfollikel und Ulvea Die In vitro-Ansammlung von C^{14} gezeichnetem Lidocain adsorbiert zu Melaningranulen war gering (12%) verglichen mit anderen Drogen wie z B Kanamycin (89%) und Chloroquin (85%) Diese autoradiographischen Resultate zeigen darauf hin daß Lidocain einen Effekt auf das Innenohr hat zusatzlich zum fruher erwiesenen Effekt auf das zentrale Nervensystem

REFERENCES

Bárány R 1935 Die Beeinflussung des Ohrensausens durch intravenos injizierte Lokalanasthetica *Acta Otolaryngol* (Stockh) *23* 201

Bernhard C G & Bohm E 1954 On the central effects of xylocaine with special reference to its influence on epileptic phenomena *Acta Physiol Scand 31* Suppl *114* 5

Dencker L & Lindquist N G 1975 Distribution of labeled chloroquine in the inner ear *Arch Otolaryngol 101* 185

Fowler E P Jr 1953 Intravenous procaine in the treatment of Meniere s disease *Ann Otol Rhinol Laryngol 62* 1186

Gejrot T 1963 Intravenous xylocaine in the treatment of attacks of Meniere s disease *Acta Otolaryngol* (Stockh) Suppl *188* 190

Hart V W & Naunton R F 1964 The ototoxicity of chloroquine phosphate *Arch Otolaryngol 80* 407

Lindquist N G 1973 Accumulation of drugs on melanin *Acta Radiol* Suppl *325*

Lin [illegible]

Potts A M 1962 The concentration of phenothiazines in the eye of experimental animals *Invest Ophthalmol 1* 522

Rahm W E Strother W F Crump J F Parker D E 1962 The effects of anesthetics upon the ear IV Lidocaine hydrochloride *Ann Otol Rhinol Laryngol 71* 116

Ward P H & Honrubia V 1969 The effects of local anesthetics on the cochlea of the guinea pig *Laryngoscope 79* 1605

Acta Otolaryngol 82 301–302, 1976

INTRAVENOUS XYLOCAINE IN THE TREATMENT OF ATTACKS OF MENIERE'S DISEASE

T Gejrot

From the Department of Otolaryngology Lasarettet Kristianstad Sweden

Abstract The effect of xylocaine on epileptic attacks suggested a study of its effect in attacks of Meniere's disease, as the symptoms can be very much alike in the two conditions The results were found to be excellent especially as regards the vegetative dysfunctions In attacks of Meniere's disease the tinnitus disappeared for 20 minutes vomiting generally disappeared for good and nausea for an hour or two Nystagmus was unaffected

Similar symptoms in attacks of Meniere's disease and epileptic seizures without loss of consciousness have prompted the use of xylocaine in antiepileptic dosage in attacks of Meniere's disease A one per cent solution without epinephrine is used in a dose of 1 mg per kg body weight administered intravenously at a rate of 6 mg per minute It is given during attacks and in periods with severe nausea

The results of this antiepileptic therapy in attacks of Meniere's disease have been strikingly good especially as regards the vegetative reactions In most cases, the nausea and vomiting disappear dramatically after about a minute and the patient can move about freely In other cases the relief is so good that the patient can sit up and take a meal without complications If the symptoms are continuous, xylocaine infusion may be used Tinnitus usually disappears for about 20 minutes, following which it gradually returns In this antiepileptic dose xylocaine has no sedative side effects and does not change the pure tone audiogram or nystagmus

As in seasickness, there are two forms of attacks of Meniere's disease Xylocaine is useful in the so-called agitatoric type with vomiting, nausea and anxiety In the asthenic type with depression nausea and decreased blood pressure, it has little or no effect In 1963, the results obtained in eleven patients treated with xylocaine in attacks of Meniere's disease were reported by Gejrot Since then, hundreds of patients have been treated with a single or repeated doses of 1–3 mg per kg body weight

Contra indications are bradycardia serious types of conduction disturbances of the heart, and a known history of hypersensitivity to local anaesthetics of the amide type There have been no complications and the results have been excellent, particularly in the agitatoric type

In epileptic seizures xylocaine acts at the cortical level The favourable effect of antiepileptic therapy on similar vegetative disturbances in agitatoric attacks of Meniere's disease seasickness and caloric stimulation suggests an epileptic equivalent

ZUSAMMENFASSUNG

Die Wirkung von intravenosen Dosen von Xylocain auf epileptische Attacke veranlaßte ein Studium der Wirkung in der Meniereschen Krankheit da die Symptome sehr ahnlich sein konnen Die Ergebnisse waren sehr gut speziell betreffs der vegetativen Storungen In den Attacken der Meniereschen Krankheit horte das Sausen wahrend 20 Minuten auf Erbrechen verschwand im allgemeinen uberhaupt und die Ubelkeit wahrend einer Stunde oder zwei Nystagmus wurde nicht beeinflußt

REFERENCES

Bernhard C G & Bohm E 1954 On the central effects of with special references to its influence on epilept c phenomena *Acta Physiol Scand* Suppl *114* 5

Gejrot T 1963 Intravenous xylocaine in the treatment of attacks of Meniere s disease *Acta Otolaryngol* (Stockh) Suppl *188* 190

— 1963 The influence of xylocaine on induced and spontaneous nystagmus *Pract Otorhinolaryngol* (Basel) *25* 361

)

Acta Otolaryngol 82 303–307, 1976

SURGICAL TREATMENT OF MENIERE S DISEASE

T Palva J Karjä and A Palva

From the Departments of Otolaryngology Universities of Helsinki and Oulu Finland

Abstract Results are reported of the effect ov various surgical methods on vertigo in Meniere s disease Minor procedures like sacculotomy or application of NaCl crystals in the round window may effect a cure but often results in deaf ears Vestibular nerve section seems to offer the best relief of vertigo while generally still preserving residual hearing

The surgical treatment of Meniere's disease is based on three principles First, certain procedures aim at improving the drainage of endolymph and thus at re establishing the spatial relations of inner ear fluids Secondly, other still conservative methods aim at partial weakening of the vestibular receptors The third principle is based upon disappointment experienced with the two above mentioned methods and attempts to discontinue totally the signals from the diseased labyrinth

Decompression procedures

The oldest method still in use is decompression of the endolymphatic sac This was initiated by G Portmann in 1927 A follow up study reported by M Portmann (1964) showed surprisingly good results relief of vertigo being obtained in about 80% Modifications of this method have utilized shunting of the endolymphatic sac into the subarachnoid space (House, 1964) or by incising the sac wall and inserting a piece of silastic sheeting into the opening (Shea 1966) but the success rate (40–50%) is similar to that obtained by simple decompression (Shambaugh Jr 1966) Of the new approaches for decompression, the blind puncture of the saccule through the foot plate was advocated by Fick (1964) This was modified by insertion of a permanent tack through the footplate to puncture the saccule as soon as a noteworthy dilatation due to endolymph increase occurred (Cody, 1969)

Partial weakening of vestibular receptors

In one of the simpler methods, a local anaesthetic is instilled into the middle ear through the drum (Ristow, 1968) Arslan (1970) inserted sodium chloride crystals into the round window niche in a less obvious attempt to cure vertigo Lastly, the conservative destruction of vestibular receptors by ultrasound was initiated by Arslan (1962) This procedure has as a target the horizontal canal in particular similar to the method of cryosurgery (Wolfson & Cutt 1971)

Destructive procedures

A time honoured procedure is the total destruction of the labyrinth in which all three semicircular canals are drilled away and the vestibule emptied Less extensive procedures aim at achieving the same goal by working through the oval window and, during the fenestration period through a fenestra in a horizontal semicircular canal Pulec (1974) added opening the internal meatus and cutting both the vestibular and cochlear nerves to total posterior labyrinthectomy

Since all these procedures, in addition to vestibular ablation, also lead to total loss of hearing, House (1961) recommended the middle fossa approach for selective cutting of the vestibular nerves while preserving the cochlear nerve

MATERIAL AND METHODS

*This series of 43 patients includes the operations performed in the period from 1964 to 1974 in Oulu University (29 patients) and from 1974 to 1975 in Helsinki University (14 patients) Ages varied from 22 to 61 years, in 5 cases the disease was bilateral, but distinctly milder in the non operated ear The patients were evaluated by a battery of pre and postoperative audiological tests including loudness balance and adaptation tests, forward and reverse Békésy tracings and stapedial reflex tests Tests for spontaneous and positional nystagmus and caloric tests were also made In the evaluation of postoperative rehabilitation, the results of these tests were compared with the patient's subjective sensations All operated patients constitute a smaller group among the total seen Conservative medical treatment and anti allergic anagement had brought no help and the pa ents were ultimately incapacitated by their vertiginous attacks

OPERATIVE PROCEDURES AND RESULTS

1 *Instillation of 4% lidocain in middle ear*

This procedure was carried out from 1 to 3 times on 3 patients Anaesthetic solution in amounts from 0 5 to 1 ml was injected through the drum under microscopic control the patient being kept in the supine position for a few hours All patients developed nystagmus towards the diseased ear and the subjective sensation of rotation varied from 2–3 hours being once 24 hours

In one patient, the weekly attacks were absent for 3 months to return in the original fashion In the other two, no clear improvement was noted Hearing was not permanently affected, nor were the caloric test results This procedure was given up because of the poor long term effect

2 *Application of sodium chloride crystals into round window niche*

Freshly made sodium chloride crystals were packed into the round window niche using endomeatal tympanotomy The crystals were covered with a small piece of gelfoam to limit them strictly to the target area All patients had spontaneous nystagmus towards the diseased ear and had a sensation of vertigo The procedure was carried out in 4 patients in one case twice at an interval of 4 months

Two of the ears in which pre operative average air conduction (AC) levels were 55 and 42 dB became deaf The first showed no change in symptoms, the other became better but was not cured of vertigo during an observation period of 4 years A third patient showing a drop in hearing from 70 to 80 dB and in discrimination from 50% to 40%, has been symptom free for 2 years The fourth patient whose hearing improved by 15 dB, has been asymptomatic for 2 years

In the caloric ENG recorded tests all patients had residual vestibular function one in the form of canal paresis and 3 in the form of directional preponderance towards the better ear

3 *Saccular puncture (Fick operation)*

This procedure was performed through endomeatal tympanotomy in 4 patients A perforation was made centrally to the footplate and the pick entered the vestibule for about 2 mm The opening was covered with a small piece of gelfoam

All ears became deaf postoperatively The caloric test showed remaining vestibular function in the form of canal paresis Three pa

tients were permanently cured of all their symptoms (observation times 8 and 10 years and one year, respectively), the fourth was symptom free for one month, but the attacks then started again with increased intensity and the labyrinth was totally destroyed (Group 5)

4 *Decompression of endolymphatic sac*

This method was used in 11 patients and consisted in mastoidectomy, desceletonizing of the labyrinth and making a blue line in the posterior canal The sac was then liberally freed of overlying bone and an opening made in its wall, where a small piece of Silastic sheeting was inserted, covered by free muscle graft

One of these 11 patients was permanently cured (observation time 5 years) The AC improved markedly, from 80 to 27 dB and discrimination from 53% to 93% A canal paresis remained unchanged in the caloric tests 5 patients improved and 5 showed no change One of the latter became deaf due to accidental opening of the posterior canal and in all except one hearing became worse during the years of follow up All patients showed unchanged reaction in the caloric test either in the form of canal paresis or directional preponderance to the opposite side The results were not influenced by the macroscopic appearance of the sac during surgery

In 3 ears with no change in symptoms, labyrinthectomy was made later (Group 5) and in one improved case vestibular neurectomy was carried out (Group 7) 7 years later the symptoms coming back again

5 *Labyrinth destruction*

This operation was carried out through the oval window in 4 ears, the vestibular contents being sucked out and the whole vestibule filled with gelfoam In 7 all semicircular canals were drilled away and the vestibule emptied 3 of the 4 ears in the former group became totally deaf as expected but in one as much as 67 dB average hearing function was retained He suffered from marked vertigo and postauricular total labyrinth destruction was carried out 5 months later All 7 ears in the latter group were deaf after surgery

These patients were observed for an average period of 5 years (range 1 to 9 years), 5 could be considered cured, and 6 improved The ENG recording of patients showed spontaneous nystagmus towards the sound side, with an average speed of the slow component of 5° sec In 7 patients this was apparent in all test positions (also sitting), while in 2 it occurred in connection with the lateral head position Two patients showed no spontaneous nystagmus, after a period of one year

6 *Labyrinth destruction combined with neurectomy*

These patients were 10 in number, and in 6 also the cochlear nerve was avulsed Postoperatively 7 had spontaneous nystagmus in all positions and 3 in the lying lateral head positon Only 3 ears have been observed over one year, and one can be classified as cured and two as improved, with some remaining imbalance Avulsion of the cochlear nerve had no permanent effect on tinnitus even though there were changes in intensity and quality of tinnitus, and tinnitus free periods occurred All patients returned to their previous work 3 to 4 months after surgery

7 *Vestibular nerve section*

This procedure was carried out in 9 patients through the middle fossa In 2 ears, the superior canal was accidentally opened and these ears became deaf In the other cases hearing improved in 3 ears, was unchanged in 3 and became worse in 1 during the observation period

All patients had spontaneous nystagmus to the unaffected side in all head positions with no reaction in caloric testing 5 patients could be considered cured and 3 greatly improved,

all having resumed their daily work 3 months after operation Two patients of the latter group have had some postoperative vertigo with fluctuation of hearing in the opposite ear

COMMENTS

It may be concluded that no permanent results are achieved by application of 4% lidocaine into the middle ear This does not mean that the method as such is wholly meaningless in cases in which more toxic drugs are used, loss of hearing, however, would be almost certain

Arslan's method using sodium chloride crystals in our hands resulted in two deaf ears and one practically deaf ear, only the fourth showed a 15 dB improvement The resulting rather grave cochlear injury is not surprising since the application site is the round window However, none of the patients lost their caloric function, even if 2 of the 4 could be considered cured and one improved of vertigo What actually happens here is unclear, but undoubtedly the permeability of the round window changes dramatically, and probably a considerable amount of perilymph is drawn out Perhaps a permanent break occurs between the cochlear and cochleo-vestibular compartments The Fick operation apparently produces also a permanent endolymph perilymph connection, and the good results in three out of four cases as regards vertigo would be worthwhile if hearing had not been lost In this respect our results disagree with those of Cody (1969), who reported only 12% hearing impairments and 70% as cured of vertigo

Our 11 decompression operations resulted in only one permanent cure and in five improved ears This contrasts clearly with Portmann's 80% good results, but accords with the experience of many others The vestibular function tests were not affected by this procedure

Labyrinth destruction, after a postoperative adaptation period of about 3 months was found to have cured vertigo in one half of the patients and relieved it greatly in the other half The latter group showed some uncertainty in walking, particularly in the dark but all could resume their previous work Vestibular nerve section gave similar results but saved hearing except in two cases of accidental superior canal opening

Avulsion of the cochlear nerve during labyrinth destruction seems to be no guarantee for the cure of tinnitus in no single case did tinnitus disappear permanently The fact that its intensity diminished in some ears argues in favour of avulsion during labyrinthectomies, but not when using middle fossa approaches

In our hands, the various minor surgical methods have been disappointing, particularly from the point of view of cochlear damage One may try Arslan's or Fick's method if one is prepared to sacrifice hearing, because many patients may be cured of their vertigo and the procedure leaves some function to the vestibular labyrinth These procedures, on the other hand, are so well tolerated and can be done under local anaesthesia, so they suit well patients with other serious illnesses Of the major procedures, vestibular nerve section effectively cures the patients of vertiginous attacks and, in the hands of an experienced surgeon, the hazards to hearing are small Destructive procedures may leave some permanent imbalance, but are the safest means of restoring the patient's working capacity

On the basis of this experience the senior author has now adopted the policy of doing a vestibular nerve section in all cases with an average hearing level of 70 dB or better and at least 50% discrimination In more advanced cases, total labyrinthectomy with nerve sectioning by Pulec's method is made since this type of patient may no longer be expected to obtain much hearing benefit in the diseased ear However, in cases suggesting bilateral involvement, vestibular nerve sectioning alone is always used

ZUSAMMENFASSUNG

Der Effekt der verschiedenen chirurgischen Verfahren auf Schwindel in Meniererscher Krankheit wird be sprochen Die kleineren Eingriffe wie Sakkulotomie oder Applikation der NaCl Kristalle auf die runde Fenster membran konnen die Schwindelanfälle beenden aber machen auf oft das Ohr taub Sektion des Nervus vesti bularis scheint am besten zu sein den Schwindel zu eliminieren und das Gehor zu erhalten

REFERENCES

Arslan M 1962 An improved technique of the ultra sonic irradiation of the vestibular apparatus by Meniere s disease *Acta Otolaryngol* (Stockh) *55* 467

— 1970 Choice of surgical procedure in Meniere s disease Proposal for a new osmotic induction method *J Laryngol Otol 84* 131

Cody D T R 1969 The tack operation for endo lymphatic hydrops *Laryngoscope 79* 1737

Fick I A van N 1964 Decompression of the laby rinth *Arch Otolaryngol 79* 447

House W F 1961 Surgical exposure of the internal auditory canal and its contents through the middle cranial fossa *Laryngoscope 71* 1363

House W F 1964 Subarachnoid shunt drainage of hydrops *Arch Otolaryngol 79* 338

Portmann G 1927 Vertigo surgical treatment by open ing the saccus endolymphaticus *Arch Otolaryngol 6* 309

Portmann M 1964 Decompressive opening of endo lymphatic sac *Arch Otolaryngol 79* 328

Pulec J L 1974 Labyrinthectomy Indications tech nique and results *Laryngoscope 84* 1552

Ristow W 1968 Zur Behandlung der Meniere Krankheit mittels temporarer Labyrinthanasthesie *Z Laryngol Rhinol Otol 47* 442

Shambaugh G E Jr 1966 Surgery of the endolymphatic sac *Arch Otolaryngol 83* 305

Shea J J 1966 Teflon film drainage of the endo lymphatic sac *Arch Otolaryngol 83* 316

Wolfson R J & Cutt R A 1971 Long term results with cryosurgery for Meniere s disease *Arch Otol aryngol 93* 483

Acta Otolaryngol 82 308–311, 1976

HEAT EXCHANGE IN CRYOSURGERY OF MENIERE S DISEASE

Experimental and Clinical Studies

S Harly, J E Aastrup and O Elbrønd

From the ENT Department University of Aarhus Aarhus Denmark

Abstract The course of the temperature in the lateral semicircular canal and the heat exchange were measured on temporal bone specimens when cooling with a cryoprobe on the lateral semicircular canal was performed The measurements showed a correlation between the *heat exchange the cooling time and the temperature in* the lateral semicircular canal In 15 patients with unilateral Meniere s disease this was utilized to achieve a controlled *temperature course during cryosurgery on the* lateral semicircular canal The effect of the treatment on the attacks of vertigo and on the hearing in these patients is reported

In cryosurgery of the labyrinth in patients with Meniere s disease (*a*) the biological reaction to a well defined temperature course—the biological aspect—should be known (*b*) it should be possible to monitor the course of the temperature through the cryo object—the physical aspects

The biological aspects of cryosurgery on the labyrinth and the consequently altered mode of reaction which eliminates the disturbances of vestibular function are not completely understood but have been studied in animals by *Ishiyama et al (1972)*

The physical aspects to be studied constitute the course of the temperature in the cryo object in this case in the lateral semicircular canal and in the facial canal

EXPERIMENTAL STUDIES

The cryo apparatus (Spembley) used for the present investigation of the physical aspects is based upon a utilization of the Joule Thomson effect, i e the cooling obtained by expansion of a gas—in this case N_2O In principle it would be possible to use other cooling systems utilizing the cooling effect of liquid nitrogen

The course of the cooling process on temporal bone specimens may be studied by placing thermocouples in the lateral semicircular canal and in the facial canal (Fig 1)

Such monitoring of the course of the cooling process by thermocouples placed in the lateral *semicircular canal and in the facial canal* is of course, not possible *in vivo*

It might perhaps be assumed that it would be sufficient to know and monitor the temperature of the cryotip carefully by means of a thermocouple incorporated in the *tip* and simultaneously measuring the cooling time An experiment on a temporal bone specimen showed that this is not sufficient (Fig 2)

Considerably lower temperatures were obtained in the canal when the heat contact between the cryotip and the bone was adequate *even though the temperature of the cryotip* was the same in both experiments viz about −70°C

Adequate control of the cooling process *in vivo* may be obtained by measuring the heat *flow through the cryotip*

A copper attachment was adapted on the tip of the probe

The attachment incorporates a thermocouple for measuring the temperature and also

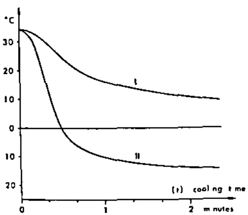


Fig 1 Recordings of temperature in I Facial canal II Semicircular canal during a cooling procedure on a temporal bone specimen Probe temperature 70°C

a differential thermocouple which renders it possible to measure the amount of the heat passing from the cryo-object into the tip of the attachment and on to the cryoprobe The differential thermocouple consists of a constantan disc soldered into a cross section 7 mm from the tip of the attachment

When a current of heat (q cal/sec) passes the differential thermocouple, a temperature difference arises across the constantan disc proportional to the current of heat and consequently an electromotive force, across the disc, proportional to the temperature difference

Thus, if the electromotive force is measured, the current of heat in cal/sec can be read directly, and if this is integrated over the cooling time, we obtain a measure of the total amount of heat, Q cal, which has passed from the cryo-object into the cryoprobe within the cooling time (Fig 3)

The course of the cryoprocess in the temporal-bone specimens was studied *partly* by measuring the course of the temperature in the lateral semicircular canal and in the *facial canal by thermocouples, partly by* measuring the heat flow and total amount of heat exchanged These cooling experiments demonstrated a correlation between the total amount of heat exchanged (Q), the temperature in the semicircular canal (T_c) and the cooling time (t), as shown in Fig 4

This correlation between temperature course and heat exchange in temporal bone specimens may be utilized for assessing the course of the cryoprocess in patients with Meniere's disease When the values measured are plotted as shown in the diagram (Fig 4),

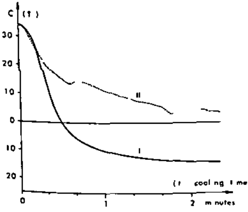


Fig 2 Recordings of temperatures in the semicircular canal (T_c) during two cooling procedures on a temporal bone specimen I Adequate thermal contact II Inadequate thermal contact between probe tip and semicircular canal

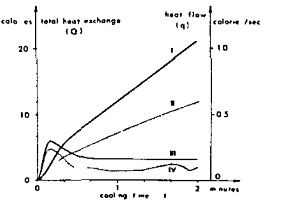


Fig 3 Recordings of total heat exchange (Q) between probe tip and semicircular canal I Adequate thermal contact II Inadequate thermal contact and heat flow (q) III Adequate thermal contact IV Inadequate thermal contact

Acta Otolaryngol 82 308–311, 1976

HEAT EXCHANGE IN CRYOSURGERY OF MENIERE S DISEASE

Experimental and Clinical Studies

S Harly, J E Aastrup and O Elbrønd

From the ENT Department University of Aarhus Aarhus Denmark

Abstract The course of the temperature in the lateral semicircular canal and the heat exchange were measured on temporal bone specimens when cooling with a cryoprobe on the lateral semicircular canal was performed The measurements showed a correlation between the heat exchange the cooling time and the temperature in the lateral semicircular canal In 15 patients with unilateral Meniere s disease this was utilized to achieve a controlled temperature course during cryosurgery on the lateral semicircular canal The effect of the treatment on the attacks of vertigo and on the hearing in these patients is reported

In cryosurgery of the labyrinth in patients with Meniere s disease, (*a*) the biological reaction to a well defined temperature course—the biological aspect—should be known, (*b*) it should be possible to monitor the course of the temperature through the cryo object—the physical aspects

The biological aspects of cryosurgery on the labyrinth and the consequently altered mode of reaction which eliminates the disturbances of vestibular function are not completely understood but have been studied in animals by Ishiyama et al (1972)

The physical aspects to be studied constitute the course of the temperature in the cryo-object in this case in the lateral semicircular canal and in the facial canal

EXPERIMENTAL STUDIES

The cryo apparatus (Spembley) used for the present investigation of the physical aspects is based upon a utilization of the Joule Thomson effect, i e the cooling obtained by expansion of a gas—in this case N_2O In principle it would be possible to use other cooling systems utilizing the cooling effect of liquid nitrogen

The course of the cooling process on temporal bone specimens may be studied by placing thermocouples in the lateral semicircular canal and in the facial canal (Fig 1)

Such monitoring of the course of the cooling process by thermocouples placed in the lateral semicircular canal and in the facial canal is, of course, not possible *in vivo*

It might perhaps be assumed that it would be sufficient to know and monitor the temperature of the cryotip carefully by means of a thermocouple incorporated in the tip and simultaneously measuring the cooling time An experiment on a temporal bone specimen showed that this is not sufficient (Fig 2)

Considerably lower temperatures were obtained in the canal when the heat contact between the cryotip and the bone was adequate even though the temperature of the cryotip was the same in both experiments viz about –70°C

Adequate control of the cooling process *in vivo* may be obtained by measuring the heat flow through the cryotip

A copper attachment was adapted on the tip of the probe

The attachment incorporates a thermocouple for measuring the temperature and also

Table II *Results upon hearing*

	Im proved	Un changed	Worse	Absent
No of patients	1	12	1	1

tients are considerably improved no change occurred in 3 patients But the observation time is relatively short—only 8–18 months

The hearing was unchanged in most of the patients

The best results seem now to have been achieved by four cryoprocedures in two dif ferent places on the lateral semicircular canal carried out in the same seance with a heat exchange of about 25–30 calories in each procedure Thereby a temperature of −18°–−20°C is obtained in the canal in each cooling period

In 3 cases, facial weakness was observed during the cooling period when the cooling time was more than 2 minutes But it dis appeared immediately on the conclusion of the cryoprocedure

We do not know whether an optimal result is obtained by aiming at such a course of the cryoprocess as mentioned above This can be disclosed only by a follow up study of the patients and by further clinical and experi mental studies—also of the biological aspects

ZUSAMMENFASSUNG

Der Temperaturverlauf im lateralen semizirkularen Gang und der Warmeaustausch wurden auf Schlafenbein praparaten wahrend Gefrieren im betreffenden semi zirkularen Gang mittels einer Kryosonde gemessen Die Messungen ergaben Korrelation zwischen Warmeaus tausch Gefrierzeit und Temperatur im lateralen semi zirkularen Gang Dies wurde bei 15 Patienten mit ein seitigem M Meniere zur Erzielung eines kontrollierten Temperaturverlaufes wahrend Kryochirurgie im late ralen semizirkularen Gang ausgenutzt. Der Effekt der Behandlung auf die Schwindelanfälle und das Hören dieser Patienten wird berichtet

REFERENCES

Ishiyama E Meyers E N & Wolfson R J 1972 Pathological and functional alterations in the ves tibular labyrinth following cryosurgery *Acta Otolaryngol* (Stockh) *74* 80

Lundquist P-G Igarashi M Wersall J Alford B R. & Wright W K 1973 Effects of ultrasound and cryosurgery on labyrinthine epithelia in the guinea pig *Adv Otorhinolaryngol Current Studies in Oto-neurology 19* 76

Acta Otolaryngol 82 312–315, 1976

THE EFFECT OF ULTRASOUND ON MENIERE'S DISEASE

H Sorensen and M Stroyer Andersen

From the University ENT Department Kommunehospitalet Copenhagen Denmark

Abstract Among 22 patients with Meniere s disease treated with ultrasonic irradiation only eight showed extinguished caloric reaction *Long term therapeutic* effect had been obtained in all the patients Histological examination of the lateral semicircular canal and the crista ampullaris from five patients previously treated with ultrasound for Meniere s disease showed the following changes (1) In the subepithelial tissue in crista ampullaris no signs of degeneration were found The nerve fibres were normal (2) The sensory cells with nerve endings and supporting cells also appeared normal (3) The secre tory cells showed pronounced cellular vacuolization and increased intercellular space (4) The lateral semi circular canal was completely obliterated with destruction of the membranous canal It is concluded that the thera peutic effect may be due to the creation of an endo-lymphatic fistula

Several papers on the therapeutic effect of ltrasonic irradiation in the treatment of enıere's disease have been published (Arslan, 1953, Angel James, 1963, Sjoberg et al , 1963, Brahe Pedersen & Sorensen, 1971) Animal experiments have been performed in order to correlate histological changes with the therapeutic effect (McLay et al , 1961, Stahle, 1964) In these, degeneration of the neuroepithelium in the ampullae, utricle and saccule was a predominant feature in the histological picture after ultrasound irradiation Generally, the labyrinthine insufficiency resulting from this degeneration is presumed to result in alleviation of the vertiginous attacks (Sjöberg et al , 1963) However, in many patients therapeutic results are obtained without severe damage to the vestibular function (Brahe Pedersen & Sorensen, 1968) The aim of the present investigation was to correlate vestibular function in patients who had received long-term therapy with the histological effect of ultrasound on the human labyrinth

CLINICAL EVALUATION

Material and method

22 patients with genuine Meniere's disease (13 women and 9 men), aged 38–76 years, obtained primary alleviation of vertiginous attacks after ultrasound irradiation At the follow-up examination 4–10 years later (mean 8 years) none of them had had relapse The results of the tests for vestibular function (spontaneous nystagmus and caloric reaction recorded by electronystagmography) are shown in Table I In 9 patients, a paralytic nystagmus could still be demonstrated Seven showed no nystagmus

Only 8 patients had extinguished caloric reaction, and in 2 it was normal In the remaining 12 the caloric reaction was diminished

Conclusion

The long term therapeutic effect of ultrasound irradiation in these patients cannot be due to a

Table I *Follow-up in 22 patients (8 years)*

Paralytic nystagmus	9
Irritative nystagmus	6
No nystagmus	7
No caloric reaction	8
Reduced reaction	12
Normal reaction	2



F g 1 Well preserved ha r cell type I surrounded by calyx *HCI* hair cell type I *SC* support ng cell

destructıon of the vest bular functıon but should be sought ın other changes ın the laby rınth

HISTOLOGICAL EXAMINATION

Materıal and method

In 5 patıents wıth typıcal Men ere s dısease wıth relapse after treatment wıth ultrasound labyrınthectomy was performed 2 10 months later At thıs operatıon the lateral sem c rcular canal and ampulla were opened and tıssue from the membranous canal and the crısta ampullarıs was removed for h stolog cal and ultrastructural exam nat on The spec men was ımmed ately fixed ın osmıum ac d

After further fixatıon ın 2 5% glutaralde hyde buffered wıth sod um cacodylate for 2–8 hours at 4 C the specımens were dehydrated ın a serıes of graded ethanol and embedded ın Epon Sect ons 1 μm ın th ckness sta ned wıth methylene blue were used for orıentatıon Ultramıcrotome sectıons were staıned w th lead cıtrate for electron mıcroscopy

Results

In the tıssue of the crısta ampullarıs the mye lınated nerve sheaths are well preserved the lamınat on ıs normal wıthout splıttıng or cleavages In the axoplasm of the rather th nly myelınated nerve fbres a few ves cles or

membrane limited cavities of non pathological significance are seen *They are technical arti* facts as no other signs of axonal degeneration are visible Also the Schwann cells *appear* normal with patchily distributed nuclear chro matin and cellular organelles No activated fibroblasts and no macrophages were found *in the tissue* The nerve endings in contact *with the hair cells* were normal and so were *the hair cells* and the supporting cells (Fig 1) The *intercellular* space was not increased *Thus* the sensory cells nerves and nerve end *ings seem resistant* to the ultrasonic irradia tion

In a patient suffering from Meniere s disease *in a* advanced stage vacuolization and exten sive swelling in the transitional epithelium surrounding the base of the crista is seen forming cell protrusions that reach far into the endolymphatic space These changes may be *regarded* as *typical* of Meniere s disease and are not a result of ultrasonic irradiation

Pronounced degenerative alterations were found in the epithelium at the base of the crista ampullaris The cells are isolated by increased intercellular space The nuclei are pyknotic and the organelles have lost their normal structures These findings are not *artifacts* as *similar* alterations are not seen in other areas of the same crista The most pronounced pathological features were how ever *found in the lateral* semicircular canal *At operation* the bony canal was in all cases found to be *completely obliterated by* whitish *fibrous tissue* and new bone formation The histological picture is dominated by dense con nective tissue containing many fibrocytes and surrounded by osteoid tissue obliterating the perilymphatic space (Fig 2) The epithel um of the duct is destroyed A fistula at least a functional one might exist in this area be tween the endolymph and the perilymph It *should* be mentioned that the superior semi circular duct was completely normal

CONCLUSION

Owing to the lack of comparative material any conclusion drown from these specimens can only be tentative Further alterations in the morphology may be due to Meniere s disease as well as to the effect of ultrasound In the interpretation of the findings the possib lity of technical artifacts must be borne in mind The sensory epithelium and the nerve-endings in the crista ampullaris as well as the nerve fibres seem to be only slightly influenced by the



Fig 2 Dense connect ve tissue completely obl erat ing the lateral sem circular canal surrounded by osteoid tissue The epithel um of the duct is destroyed

radiation The degenerative alterations in the transitional and secretory epithelium around the base of the crista may influence the labyrinthine hydrops and thereby contribute to the claimed therapeutic effect of ultrasound However, the pathological alterations in the connective tissue in the lateral semicircular canal are the most pronounced findings The massive destruction of the normal anatomy in this area may be the morphological basis for an endolymphatic fistula and this may be the fundamental effect of the ultrasonic irradiation

This is in agreement with the theory of the analogous effect of cryosurgery (House, 1966), by which a fistula is also supposed to be created, and which explains the therapeutic results in patients with preserved vestibular function It is also in agreement with experimental endolymphatic hydrops and its relief by interrupting the lateral semicircular duct (Konishi & Shea, 1975)

ZUSAMMENFASSUNG

Von 22 mit Ultraschall behandelten Patienten mit Memeres Krankheit zeigten nur 8 Aufhören der caloric Reaktion Langanhaltender klinisch therapeutischer Effekt wurde bei allen diesen Patienten erreicht Die histologische Untersuchung des lateralen Bogengangkanales sowie der Crista ampullaris von 5 mit Ultralaut behandelten Patienten zeigten folgende Veranderungen Das subepitheliale Gewebe der Crista ampullaris zeigte keinerlei degenerative Veranderungen Auch die Nervenfasern waren normal Ebenso waren die Sinneszellen mit Nervenendigungen und Stutzzellen normal Die sekretorischen Zellen zeigten deutliche Vacuolisierung und Zunahme der intercellularen Raume Der laterale Bogengangskanal war total oblitenert mit Zerstorung des membranosen Kanales Man konkludiert daß der therapeutische Effekt wahrscheinlich auf der Bildung einer endolymphatischen Fistel beruht

REFERENCES

Angel James J Freundlich H F Bullen M A Wells P N T & Williams D P C 1963 The physical and biological properties of ultrasound and clinical experience *Acta Otolaryngol* (Stockh) Suppl *178* 134

Arslan M 1953 Treatment of Meniere s syndrome by *direct application of ultrasound waves to the vestibular system* *Proc Vth Int Congr Otorhinolar* Amsterdam

Brahe Pedersen C & Sørensen H 1968 Ultralydbehandling af morbus Meniere *Ugeskr Læger 130* 83

— 1971 Labyrinthectomy and ultrasonic irradiation in Meniere s disease *Dan Med Bull 18* 66

House W F 1966 Cryosurgery in Meniere s disease *Arch Otolaryngol 84* 616

Konishi S & Shea J J 1975 Experimental endolymphatic hydrops and its relief by interrupting the lateral semicircular duct in guinea pigs *J Laryngol Otol 89* 577

McLay K Flinn M & Ormerod F C 1961 Histological changes in the inner ear resulting from the application of ultrasonic energy *J Laryngol Otol 75* 345

Sjoberg A Stahle J Johnsen S & Sahl R 1963 Treatment of Meniere s disease by ultrasonic irradiation *Acta Otolaryngol* (Stockh) Suppl *192*

Stahle J 1964 Some effects of ultrasound on the inner ear *Acta Otolaryngol* (Stockh) Suppl *192* 192

Acta Otolaryngol 82 317–324, 1976

NERVE FIBERS IN THE HUMAN ORGAN OF CORTI

Y Nomura

From the Department of Otolaryngology University of Tokyo Tokyo Japan

(Received January 19 1976)

Abstract In the human cochlea the following characteristics of nerve fiber arrangements were observed

1 Where there was only the basilar fiber in the tunnel of Corti the internal and tunnel spiral bundle were not observed In such cases the number of nerve fibers in the external spiral bundle was small

2 In the tunnel of Corti the basilar fibers (afferent) took a course at the base and crossing the tunnel slanting to the basal end

3 The medial fibers were present with the internal spiral bundle and tunnel spiral bundle These fibers are possibly efferent

4 The external spiral bundle increases its fiber count when internal and tunnel spiral bundles were present in the organ of Corti

5 The external spiral bundle was composed of the basilar and the medial fibers The former were less and the latter much more numerous in the bundle

6 85–90% of the afferent fibers went to the internal hair cells while the remainder went to the external hair cells

7 The nerve fibers were found in the organ of Corti lateral to the external spiral bundles The fibers were running in the Hensen s and Claudius cell area

8 In some cases medial fibers showed a torpedo-like deformity in the tunnel of Corti When this was observed it was found distributed throughout the entire cochlea Hearing disorders due to this pathology of the efferent fiber was suggested

Analysis of the sound transmitted into the cochlea and the coding of its impulses to the eighth nerve are accomplished in the periphery of the hearing sense organ under the regulation of nerve fibers Complex arrangements of nerve fibers in the human organ of Corti are necessary to control the neural mechanism of hearing and to maintain the integrity of the cochlear function The sensori neural unit in the organ of Corti is composed of afferent and efferent fibers and sensory cells Recent advances in electron microscopic studies have provided new data in this area In addition, it has been postulated that the autonomic nervous system plays an important role in the hearing mechanism

Data on the human hearing mechanism, however, have not been sufficient, as post-mortem autolysis makes the observation of fine structures difficult

The purpose of this paper is to discuss the distribution pattern of the nerve fibers in the osseous spiral lamina as well as the organ of Corti in the human cochlea using histological and histochemical staining techniques Pathological changes in the nerve fibers are also discussed

MATERIALS AND METHODS

Human temporal bones were removed at autopsy and fixed in 10% formalin solution The bone was decalcified with 5% solution of trichloracetic acid After neutralization and washing in water, the bone was dissected under the operating microscope The osseous spiral lamina and the basilar membrane with organ of Corti were dissected out of the membranous labyrinth Holmes' silver staining method was applied for staining of the tissue A stepwise procedure of the staining was mentioned in a previous report (Nomura & Kirikae, 1967)

A portion of this paper was read at the First International Workshop on Sensorineural Deafness on December 2 1974 in Nashville Tenn USA

This research was supported by the Research Grant for Specific Diseases of the Ministry of Health and Welfare Sudden Deafness Research Committee of Japan

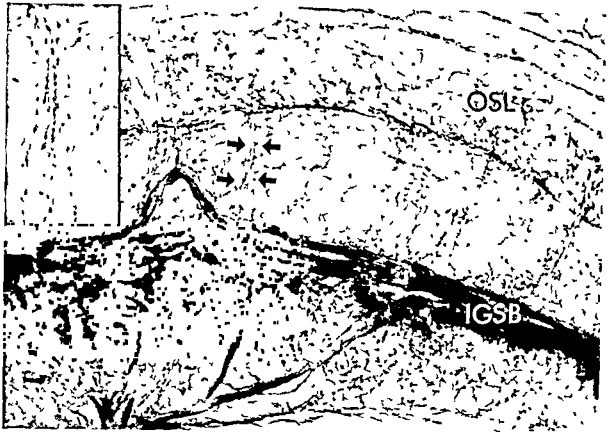


Fig 1 Efferent fiber arrangement in the human cochlea Intensely stained bundles are intraganglionic spiral bundle (*IGSB*) From here the fiber goes to the osseous spiral lamina (*OSL*) and forms many spiral bundles A nerve bundle contains both stained (efferent) and unstained (afferent) fibers *SG* spiral ganglion cells in the Rosenthal's canal Inset is a high power view of a bundle indicated by arrows Note beads in the nerve fiber AChE stain

) Adam's osmium tetraoxide α-naphthylanine (OTAN) method was employed to demonstrate the myelin sheath of nerve fibers in the osseous spiral lamina

The efferent nerve fiber was visualized histochemically by demonstrating acetyl cholinesterase (AChE) activity Karnovsky-Root's method was employed in this staining

Stained specimens were dehydrated in graded alcohol, cleared in xylene, and mounted in synthetic resin Observation was made under the light microscope

FINDINGS

Osseous spiral lamina

There are two fiber arrangements in the human osseous spiral lamina, radial and spiral In the normal cochlea, an individual fiber could not be traced in the osseous spiral lamina because of the great density of nerve fibers However it was easy to trace fibers in the cochlea of the aged, because many nerve fibers were missing, particularly in the lower basal turn

The spiral bundles entered into the osseous spiral lamina from the intraganglionic spiral bundle (Fig 1) They took a spiral course apicalwards While running, these bundles made many crossings where some nerve fibers moved from the original bundle to another, as in the fiber arrangement in the optic chiasma (Fig 2) Thus, a fiber which entered into the osseous spiral lamina of the basal turn takes a long way to the upper turn by changing spiral bundles Application of the histochemical technique revealed that the spiral bundles



Fig 2 A crossing of efferent spiral bundles in the osseous spiral lamina Nerve fibers partially interchange the bundle like fiber arrangement in the chiasma Holmes stain

were positive for AChE The fiber goes to the organ of Corti through the habenula perforata In presbyacusis the efferent nerve fibers also degenerate and disappear in the lower basal turn

We had a case in which the spiral bundles were completely missing bilaterally

A 78 year old deaf mute died of pneumonia His audiogram showed sensori neural hearing loss of 80 dB bilaterally

The cochleas were stained by the OTAN method There was a marked loss of radial fibers in the basal turn but many radial fibers remained in the upper turn However no spiral bundles were present throughout the whole cochlea (Fig 3) Severe strial atrophy (or agenesis) was found in the cochlea

Organ of Corti

The myelinated nerve fiber loses its myelin sheath before passing through the habenula perforata The unmyelinated nerve fiber makes spiral and radial fiber arrangements in the organ of Corti

In the tunnel of Corti two radial fibers were recognized the basilar fiber and the medial fiber The basilar fiber crosses the tunnel at the base while the medial fiber crosses high in the tunnel The basilar fiber crosses the tunnel slanting basalward and the medial fibers apicalward The medial fiber has more bead formations than the basilar

A cochlea of an 88 year-old man showed only the basilar fiber remaining in the basal turn where the number of nerve fibers in the external spiral bundle was extremely small Furthermore there was no inner spiral bundle or tunnel spiral bundle These spiral bundles in the organ of Corti coexist with the medial fiber in the tunnel of Corti

In a developing cochlea of a 6-month old embryo only the basilar fiber was recognized